UNIVERSITY OF CAPE TOWN

# The Relationship between Immunization and Food Allergy and Sensitisation in South African Children

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## Preamble

## Declaration

I, *Nomathamsanqa Ndhlovu,* hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signature: ndhlovu

Date: 06 February 2017

## Dedication

*To a wonderful Saviour…who brought me this far and gave me the opportunity to do an MPH.*

## Abstract

The prevalence of food allergies is higher in children compared to adults and it is increasing. The factors that influence food allergies in children are not clear. In light of the hygiene hypothesis, vaccinations may contribute towards to a predominant allergen specific response or exposure to the virus or microbe in the vaccine may decrease the risk for allergy. Previous studies have shown that the effect of vaccinations on food allergy and food sensitisation varies. Therefore, the aim of this study is to determine if a relationship exists between vaccinations and food allergies and food sensitisation in children in the first 18 months of life who live in urban Cape Town and in rural Mqanduli in the Eastern Cape. Secondary data analysis of an observational cross sectional study was carried out which involved univariate logistic regression to calculate odds ratios between self-reported immunisation status and food sensitisation and food allergy at a 95% confidence interval in children between 12 and 36 months of age. The same method was employed to investigate the relationship between immunisation and atopy. Multivariate analysis was utilised to adjust for potential confounders. Food sensitisation and food allergy were determined through skin prick tests (SPT) and oral food challenges respectively. The results indicate that, the number of participants positive for food sensitisation and allergy, eczema, hay fever and asthma were significantly greater in the urban sample (n= 708) compared to the rural sample (n= 400) ($P<0.05$). Further, in 708 urban children, those who had a BCG vaccine at birth were 0.05 (OR 0.05; 95% CI: 0.004 - 0.6) times less likely to have an SPT $\geq$ 7mm. The BCG unvaccinated cohort consisted of three individuals. There were no other significant associations between childhood vaccinations and food sensitization at SPT $\geq$1mm, $\geq$3mm and $\geq$7mm. There was no significant association between vaccinations and food allergy or other forms of atopy. In

conclusion, there was very little evidence of an association between BCG vaccination in children and food allergic sensitisation or food allergy. However, in a small subgroup, there was evidence in an association between BCG and SPT $\geq$7 mm.

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The current study was secondary data analysis of data collected through the South African Food sensitisation and Food Allergy (SAFFA) study. The following individuals were involved in the SAFFA study and would be co-authors if the manuscript of this research were published.

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## List of Abbreviations

| Abbreviations | |
|---|---|
| HREC | Human Research Ethics Council |
| IgE | Immunoglobulin E |
| ISAAC | International Study on Asthma and Allergies in Childhood |
| OR | Odds ratio |
| RCT | Randomised control trial |
| REF | Reference |
| SAFFA | South African Food Sensitisation Food Allergy |
| SPT | Skin Prick Test |
| TH | T helper |
| YRS | Years |
| WKS | Weeks |
| Vaccinations | |
| BCG | Bacillus Calmette Guérin |
| DTaP-IPV/Hib | Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b |
| DTP | Diphtheria tetanus whole pertussis |
| HBV | Hepatitis B Vaccine |
| MV | Measles vaccine |
| OPV | Oral Polio Vaccine |
| PCV | Pneumococcal conjugate vaccine |
| RV | Rotavirus vaccine |

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# PART A

## Protocol

# Part A: Protocol

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# The relationship between immunisation and food allergy and sensitisation in South African children

## Purpose of the study

The aim of this research is to determine if an association exists between vaccinations and food sensitisation and food allergy in children in the first 18 months of life who live in urban Cape Town and rural Mqanduli in the Eastern Cape. The hypothesis is that immunisation is correlated with food sensitisation and food allergy in children from South Africa.

## Rationale & background information

Immediate type food allergy is defined by the World Health Organisation (WHO) as the development of an immune reaction within an hour to a specific food consumed (WHO and INFOSAN, 2006). A food allergy is either Immunoglobulin E (IgE) mediated or non IgE mediated (WHO and INFOSAN, 2006). IgE mediated food sensitization can be determined through a skin prick test (SPT) or through the identification of allergen specific IgE antibody in an individual's blood (Gray et al., 2014). While the presence of food sensitisation is a pre-requisite for a diagnosis of food allergy, only some people with food sensitisation will go on to develop food allergy (WHO and INFOSAN, 2006). IgE mediated food allergies are confirmed through food challenge testing (Gray et al., 2014).

It is estimated that 1-10% of people suffer from a food allergy worldwide (Rona et al., 2007). When the prevalence of common food allergies was investigated in Europe, lifetime prevalence was found to be; 0.33-6% for cow's milk, 0.23- 3.61% for egg allergy, 0.30- 3.61% wheat allergy, 0.26-3.16% soy allergy, 0.22- 8.58% for peanut allergy, 0.45-1.32% for tree nut allergy, 0.62- 2.17% for fish allergy and 0.70-1.31% for shellfish allergies (Nwaru et al., 2014).

Food allergy prevalence was determined through self-reporting, open food challenge (OFC), skin prick testing (SPT) or specific immunoglobulin E (IgE) testing (Nwaru et al., 2014).

The prevalence of some food allergies is higher in children compared to adults. A meta-analysis found that children less than one year old had cow's milk and egg allergy prevalence of 2% and 0.71% respectively compared to 0.61% and 0.23% in adults (Nwaru et al., 2014). Many children outgrow food allergies (Kattan, 2016), over ten years the cumulative incidence of food hypersensitivity decreased by 3.2% in a prospective study (Venter et al., 2016). Therefore, the prevalence of food allergy is higher in children.

The prevalence of food allergies amongst children in South Africa is not well described. One study reported food allergy prevalence amongst children between 6 months and 10 years with severe atopic dermatitis to be 40% (Gray et al., 2014). Food allergy was confirmed using OFC once sensitisation had been established using SPT, IgE blood testing and clinical history (Gray et al., 2014). Atopic dermatitis is a type of eczema which is an acknowledged risk factor for food allergies (Gray et al., 2014). Recently, results were published indicating a prevalence of food sensitization (SPT$\geq$1mm) to be 12.3% and food allergy to be 2.5% amongst 512 urban Cape Town children between 12 and 36 months of age (Basera et al., 2015). With regards to food allergies, the study reported a prevalence of food allergies confirmed through an oral food allergy test to be 1.8%, 0.2%, 1.2% and 0.2% for egg, cow's milk, peanut, and fish respectively (Basera et al., 2015).

**Table A1: South Africa vaccine schedule**

| Time | Vaccination | Time | Vaccination |
|---|---|---|---|
| Birth | BCG | 14 weeks | RV(2) |
| | OPV (0) | | DTaPIPV/HiB(3) |
| 6 weeks | OPV (1) | | HBV(3) |
| | RV (1) | | PCV7 or 13 (2) |
| | DTaPIPV/HiB (1) | 9 months | Measles vaccine |
| | HBV (1) | | PCV 7 or 13 (3) |
| | PCV7 or 13(1) | 18 months | DTaPIPV/HiB (4) |
| 10 weeks | DTaPIPV/HiB (2) | | Measles Vaccine |
| | HBV (2) | | |

Vaccines are offered routinely to children in South Africa (Table A1). Vaccines may act as modifiable protective factors or risk factors for food sensitisation and food allergies in South African children. It has previously been hypothesised that a lack of early childhood exposure to microbes and infections in the modern era has led to an increase in atopic disorders (Obihara and Bardin, 2008). This phenomenon is coined as the "hygiene hypothesis" (Strachan, 1989). Following from this theory, research has been carried out to determine whether attenuated infectious agents in vaccinations may reduce or increase the incidence of atopic disorders (Obihara and Bardin, 2008).

### BCG vaccine

An inverse relationship between the Mycobacterium Tuberculosis Bacillus Calmette–Guerin (BCG) vaccine and atopic diseases has been identified in studies carried out in different parts of the world (Obihara and Bardin, 2008). In Guinea Bissau, children who had a BCG vaccination scar and documentation confirming BCG vaccination had a 59% (OR 0.41; 95% CI: 0.22- 0.76) decreased odds for atopy (any SPT wheel ≥2) (Aaby et al., 2000). Similarly, a study showed that children who had a BCG vaccination scar had a 58% (OR 0.42; 95% CI: 0.19–0.94) reduced odds of atopy (any SPT wheel ≥3mm to egg white, peanuts, cow's milk, house dust mites or

cockroach) compared to those without a scar (Kiraly et al., 2013). The same study found that the time of BCG vaccination, early or delayed, was not significantly associated with atopy (Kiraly et al., 2013). These studies point to the possibility of childhood vaccines being protective for atopy, food sensitisation and allergies in other populations in Africa. Contrastingly, in 2014, a systematic review and meta-analysis reported no significant association between BCG and self-reported asthma, eczema, rhinoconjunctivitis and other common allergies in children between 13 to 17 years of age (Linehan et al., 2014). Different results from previous studies looking at similar associations between vaccines and allergy-related outcomes indicate the importance of carrying out further research that will add towards reaching definitive conclusions on allergies.

### DTP vaccine

The Diphtheria, Tetanus and Pertussis (DTP) vaccine which is administered to infants in South Africa at 6, 10, 14 and 72 weeks (Amayeza-Information-Services, 2015) may be a risk factor for atopy, protective against atopy or not associated with atopy disorders. A British study found an association between diphtheria, polio, pertussis, and tetanus (DPPT) vaccination and incident diagnosed asthma (OR 10.33; 5.36- 19.91) and incident diagnosed eczema (OR 7.51; 5.27- 10.70) in infants in the first 6 months of life, when controlling for GP visits (McKeever et al., 2004). Atopic dermatitis (eczema) is a risk factor for food allergies (Heratizadeh et al., 2011) and this may point to DTP as a possible risk factor for food sensitisation and allergies in children. Contrastingly, in a Swedish study, no significant association was identified between asthma medication use and DTP (Vogt et al., 2014). This study investigated asthma in adolescent participants who were exposed to DTP as children (Vogt et al., 2014). Delaying a vaccination may be protective for atopy. This is demonstrated by a cross sectional study carried out in Melbourne, Australia, that reported DTaP administered with a month delay was

associated with a reduced risk of doctor diagnosed eczema (OR 0.57; 0.34- 0.97, p=0.04) compared to when the vaccine was administered at the standard time in one year old infants (Kiraly et al., 2016). These studies indicate that DTaP and DPPT have the possibility to be a risk factor for atopy, protective against atopy or not associated with atopy disorders. Inconclusive results on the association and type of association warrants further research to determine the relationship between DTP and atopy including food allergies and sensitisation.

### MMR vaccine

A measles vaccine at the time of the study was administered at 9 months and 18 months to children through the public health sector in South Africa (Amayeza-Information-Services, 2015), changed in 2016 to 6 and 12 months (NICD, 2016). In the Faroe Islands, the measles mumps and rubella (MMR) vaccine was found to be significantly associated with a reduced odds of 0.16 (0.05 - 0.53) of getting clinically examined asthma in 13 year old children (Timmermann et al., 2015). An additional study found the opposite association where MMR significantly increased the risk of incident diagnosed eczema (hazard ratio 4.61; 3.15- 6.74) and asthma (hazard ratio 3.51; 2.42- 5.11) (McKeever et al., 2004) in infants in the first 6 months of life. It is not known which component of MMR may be responsible for reducing or increasing the risk of asthma and eczema.

The studies described above indicate associations between vaccinations and atopy in first world countries and one indicating an association in a West African country (McKeever et al., 2004, Kiraly et al., 2013, Timmermann et al., 2015, Aaby et al., 2000). Determining if similar associations exist for a South African population will provide important insights into the factors associated with allergies in South Africa. Although the studies do not provide information on the association between food sensitisation or allergy and immunisation, the

association found between vaccines and atopy provide grounds for research into vaccines and food sensitisation and allergy.

The South African Food sensitisation Food Allergy (SAFFA) study is being carried out to investigate the prevalence of IgE mediated food allergy in South African children (Levin et al., 2013). Furthermore this study aims to investigate modifiable and non-modifiable risk factors and protective factors for food allergies and food sensitisation in children from South Africa (Levin et al., 2013). Thus, as a subpart of the SAFFA study, the aim of this study is to determine if a relationship exists between food allergies and vaccinations administered to children in the first 18 months of life who live in urban Cape Town and rural Mqanduli, in the Eastern Cape.

## Study goals and objectives

The aim of the research is to determine if a relationship exists between vaccinations and food allergy and food sensitisation in children in the first 18 months of life who live in urban Cape Town and in rural Mqanduli in the Eastern Cape.

Objectives

1. To describe the pattern of immunisation coverage in South African children
2. To compare the pattern of immunisation coverage in rural and urban South African children
3. To investigate the relationship between immunisation (appendix 1) administered up until 18 months and food allergy and food sensitisation (egg, cow's milk, soy, wheat, fish, peanut and hazel nut) in children between 12 and 18 months.
4. To investigate the relationship between immunisation (appendix 1) administered up until 18 months and self-reported asthma, hay fever and eczema in children between 12 and 18 months.

## Methodology

### Study Design

The study design will be an observational cross sectional study carried out using secondary data analysis at the Red Cross War Memorial Hospital in Cape Town. All children tested for food sensitisation will be selected from the SAFFA database and vaccinations previously administered will be assessed.

### Power to carry out study

In order to carry out this study, the probability that the tests carried out will result in a significant difference at a 95% significance level ($\alpha$= 0.05), the power of the test will be set at 80% (1- $\beta$). To calculate the power to test whether immunisation is significantly associated with food sensitisation, the immunisation coverage in children with food sensitisation and allergy compared to those without in South Africa, will be required. South African immunisation coverage ranges from 55.2%-100% nationwide and coverage is 92.6% in urban Cape Town (Massyn et al., 2013). There is no data indicating the coverage of vaccination amongst children who are sensitised or allergic to foods in South Africa. Therefore, an estimated figure for the exposure of vaccination will be used from the Abby et al., (2000) paper, which reports that immunisation coverage in children with atopy is 73% (Aaby et al., 2000). Further, the Urban Cape Town cohort of the SAFFA study consists of a sample size of 668 where 12.3% tested positive from a SPT with a reactive wheal $\geq$1mm, 9.6% tested positive from a SPT with a reactive wheal $\geq$ 3mm and 4.5% tested positive from a SPT with a reactive wheal $\geq$ 7mm (Basera et al., 2015) (Table A2). Power calculations indicate that there is enough power to perform tests looking at vaccine exposure in sensitised and non-sensitised for those found positive for a SPT greater than 1mm and 3 mm (Table A2). However, there isn't enough

power (68.3%) to detect a significant association ($\alpha$= 0.05) for participants with a SPT reactive wheal greater than 7mm (Table A2).

**Table A2: Table showing variables for URBAN cohort utilised to calculate power to investigate association between immunisation and different levels of sensitisation**

| SPT reactive wheal size | ≥ 1mm | | ≥ 3mm | | ≥ 7mm | |
|---|---|---|---|---|---|---|
| N = 668 | sensitised | Non sensitised | sensitised | Non sensitised | sensitised | Non sensitised |
| Total numbers (n total= 668) | 82 | 586 | 64 | 604 | 30 | 638 |
| Estimated immunisation coverage (Exposure) | 73% | 90% | 73% | 90% | 73% | 90% |
| Power to find a difference % | 95.9% | | 91.7% | | 68.3% | |

Urban cohort

The estimated immunisation coverage in the OR Tambo district where Mqanduli is located is approximately 75% in under 1 year olds (Massyn et al., 2013). The rural Eastern Cape cohort includes 400 participants and the number of participants who are positive for food sensitisation (reactive wheal ≥ 1mm) is 18 (4.5%). If an immunisation coverage of 73% (Aaby et al., 2000) is used as an estimate for those immunised in the food sensitised participants, the study is insufficiently powered (3.4%) for an investigation into the relationship between food sensitization and the exposure of immunisation in the rural cohort (Table A3). However, the prevalence of immunisation in food sensitised children from rural areas in South Africa is unknown. If it is found that immunisation coverage is 40% or less in the sensitised rural participants, then the investigation will be adequately powered (Table A3). Therefore this part of the study may nevertheless be carried out due to it being the first of its kind being performed in South Africa.

**Table A3: Table showing variables for RURAL cohort utilised to calculate power to carry out analysis between sensitisation and immunisation for different levels of immunisation coverage in sensitised participants**

| n total = 400<br>Prevalence | Sensitised (SPT ≥ 1mm)<br>4.5% (n = 18) | Non sensitised<br>(n= 382) | Power % |
|---|---|---|---|
| Estimated immunisation coverage % | 95 | 75 | 28.9 |
| Estimated immunisation coverage % | **73** | **75** | **3.4** |
| Estimated immunisation coverage % | 65 | 75 | 11.9 |
| Estimated immunisation coverage % | 40 | 75 | 82.9 |

Rural cohort

## Characteristics of the study population

A group of 708 urban Cape Town participants and 400 rural Eastern Cape participants will be utilised for the study. Study participants will comprise of children between 12-36 months of age from registered crèches in metropolitan Cape Town and from communities surrounding health centres in Mqanduli in rural Eastern Cape.

**Inclusion criteria for participants**

- Participants who are between 12 and 36 months. Children who are less than 18 months will not be assessed for vaccines at 18 months.
- Tested for food sensitization by means of a skin prick (SPT ≥1mm, ≥3mm and ≥7mm) test to the following foods; egg, cow's milk, soy, wheat, fish, peanut and hazel nut.
- If sensitised tested for food allergy to at least one; egg, cow's milk, soy, wheat, fish, peanut and hazel nut, through an oral food challenge.
- Participants with self-reported information on asthma, hay fever and eczema

**Exclusion criteria**

- Participants with missing or unclear information for food allergy and food sensitisation tests.
- Participants with missing or unclear information on vaccination status.

### Recruitment and enrolment

Secondary data analysis will be employed for this study. Thus, data for participants will be collected from the database of the SAFFA study. The SAFFA study acquired its data through administering a questionnaire to parents and guardians and performing skin prick tests and oral food challenge tests on participants. All children with data in the SAFFA database will be included unless they are deemed to be ineligible by the principal investigator and supervisor in terms of the inclusion and exclusion criteria outlined above.

### Research procedures data collection methods

As mentioned previously, the SAFFA study collected data using questionnaires and confirmed food allergy and sensitization through clinical history and food allergy and sensitization tests. The current investigation will not collect data directly from participants because secondary data analysis will be carried out.

## Data safety and monitoring

### Data analysis

Describe the pattern of immunisation coverage in South African children

- Exploratory data analysis will be performed through the use of descriptive measures, frequency distributions and graphs such as bar graphs, pie charts and histograms for each vaccine.

To compare the pattern of immunisation coverage in rural and urban South African children

- Exploratory data analysis will be performed through the use of descriptive measures, frequency distributions and graphs such as bar graphs, pie charts and histograms for each vaccine for the rural and urban cohort separately.

To investigate the relationship between immunisation and food allergy and food sensitisation

- Chi squared test between immunisation and food sensitisation will be carried out to investigate a possible association.
- As this is a cross sectional study prevalence ratios will be calculated to investigate the relationship between immunisation and food allergy and food sensitisation.
- Multivariate logistic regression analysis will be utilised to investigate the risk or protection of food sensitisation when exposed to various vaccinations while accounting for potential confounders.

To investigate the relationship between immunisation and self-reported asthma, hay fever and Eczema.

- Chi squared test between immunisation and self-reported asthma, hay fever and eczema will be carried out to investigate a possible association.
- Prevalence ratios will be calculated to investigate the relationship between immunisation and self-reported asthma, hay fever and eczema.
- Multivariate regression analysis will be utilised to investigate the risk of asthma, hay fever and eczema when exposed to various vaccinations while being conscious that asthma, hay fever and eczema were self-reported measures.

## Ethical considerations

The study seeks to investigate a phenomenon in a vulnerable population group, which are children between 12 and 36 months of age. Therefore risks, benefits and confidentiality are important factors to consider in this research. The SAFFA study on children between 12- 36

months was approved by the human ethics committee at the University of Cape Town. Ethics approval was received on January 2013. For the current study “The relationship between immunisation and food allergy and sensitisation in South African children”, ethics approval to perform secondary data analysis will be required.

### Risks

The study involves secondary data analysis and the risks for participants will be low. The SAFFA database does not contain names and recognisable personal patient information. Unique numerical identifiers are used to differentiate participants on the database. The names of the participants and any other direct personal information can only be accessed by the principal investigator of the SAFFA study. However, it is possible that while the study is being carried out, one might need to refer to a participant questionnaire for clarification of a certain variable and in the process, come across personal information. If this happens, personal identifying information will not be included and the risk in breaching confidentiality will be kept at a minimum.

### Benefits

The participants of this study will not directly benefit from this study, however the knowledge gained form the study has positive implications for children. At a societal level, the study will contribute to the knowledge and understanding of risk factors or protective factors associated with food allergy and sensitisation among South African children. This will assist health workers to predict risks or protective elements and assist with preventing food allergies and sensitisation associated with vaccinations. Further, this research will allow for patients to be advised on whether vaccines have an association with food sensitisation and allergy.

### Informed consent process

Informed consent was obtained from guardians and parents for all participants in the SAFFA study. Therefore all study participants loaded on the SAFFA study database have provided informed consent for the relationship between vaccines and food sensitisation and allergy to be carried out. In the current study, information from the database will be used and there will be no patient contact between the researcher and the participants.

### Privacy and confidentiality

To ensure respect for participants in this study, confidentiality of participation in research will be maintained throughout the entire study and thereafter. Names and personal information of participants can only be accessed by the principal investigators of the SAFFA study. This information will not be available on the database, rather anonymous numerical identifiers will be used to identify participants. In this way the confidentiality of participants will be protected. Further, all electronic files used in the current study will be password protected.

## Dissemination

The results of this research will be published as a journal article to share the information obtained with the larger allergy clinical and scientific research body. Further, the findings of this research will be made available to those in the department of paediatrics and child health, to contribute towards better knowledge on modifiable factors for paediatric food allergies and sensitisation in South Africa.

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# PART B

## Literature Review

# Part B: Literature Review

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# LITERATURE REVIEW

## Introduction

### Food allergy

Food allergy is defined as an immune mediated individual hypersensitivity reaction to food (WHO and INFOSAN, 2006). Subtypes of food allergy include IgE mediated food allergy which occurs within two hours of a specific food consumed and non-IgE mediated or "delayed type" food allergy (ASCIA, 2016, WHO and INFOSAN, 2006). While the presence of food sensitisation is a pre-requisite for a diagnosis of IgE mediated food allergy, only some people with food sensitisation will go on to develop food allergy (WHO and INFOSAN, 2006).

It is estimated that 1-10% of people suffer from a food allergy worldwide (Rona et al., 2007). When the prevalence of common food allergies was investigated in Europe, lifetime prevalence was found to be; 0.33-6% for cow's milk, 0.23- 3.61% for egg allergy, wheat allergy, 0.26-3.16% soy allergy, 0.22- 8.58% for peanut allergy, 0.45-1.32% for tree nut allergy, 0.62-2.17% for fish allergy and 0.70-1.31% for shellfish allergies (Nwaru et al., 2014). Food allergy prevalence was determined differently using self-reporting, OFC, SPT or specific IgE testing (Nwaru et al., 2014).The prevalence of some food allergies is higher in children compared to adults. A meta-analysis found that children less than a year old had cow's milk and egg allergy prevalence of 2% and 0.71% respectively compared to 0.61% and 0.23% in adults (Nwaru et al., 2014).

The prevalence of food allergies has been increasing over time (Kulis et al., 2015). In China, a developing country, the prevalence of food allergy increased from 3.1% in 1999 to 7.7% in 2009 ($p = 0.017$) in 0- 24 month old children tested through oral food challenges (Hu et al., 2010). Similarly, the prevalence of "food allergy" as a discharge diagnosis in hospitalised patients, in the USA, has increased significantly ($p < 0.001$) over time from periods 1998 and

2000 to 2001 and 2003 to 2004 and 2006 (Branum and Lukacs, 2009). Additionally, data from the National Health Survey in the USA, indicated an increasing trend (P=0.01) in self-reported food allergy between 1997 and 2007 (Branum and Lukacs, 2009). In Australia, a similar trend was observed, where food induced anaphylaxis admissions increased between 1994 and 2004 (P<0.001) (Liew et al., 2009).

### Atopy

Atopy is the familial or personal tendency to develop IgE antibodies in response to innocuous environmental antigens and to elicit allergic symptoms such as atopic dermatitis, allergic rhinitis, asthma and food allergy (Kulis et al., 2015, Daschner et al., 2008, Valenta et al., 2015). There is therefore a strong link between food allergy and asthma, allergic rhinitis and eczema. Atopy is a risk factor for food allergies and the presence of eczema confers additional risk (Gray et al., 2014). A cohort study carried out in Paris indicated that 6% of infants who had atopic dermatitis were sensitised (≥ 0.35 kU/l of specific IgE) to at least one of the following foods; cow's milk, hen's egg, peanut, soy, fish, and wheat (Just et al., 2014). Although this doesn't indicate a causal association, it does indicate that in infants with atopy, food sensitization frequently co-occurs. Furthermore, a Japanese study found that the risk of wheezing, eczema and rhinoconjunctivitis occurring in the last 12 months, increased by 12.8 to 71.4 times in primary school children who had self-reported food allergies for egg, cow's milk, wheat and crab, compared to those who didn't (Kurosaka et al., 2011). Self-reported allergies data was collected through completion of the International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire by family members of the children (Kurosaka et al., 2011).

## Hygiene hypothesis

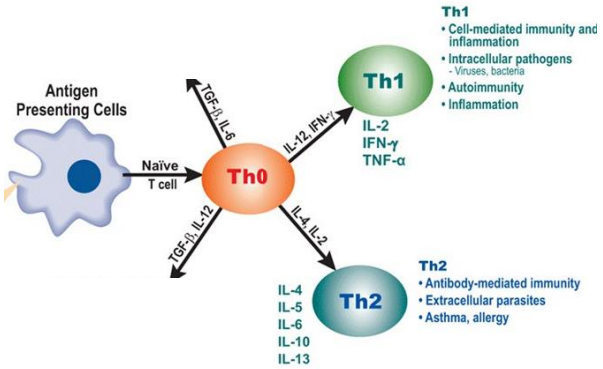
The hygiene hypothesis is based on the thinking that a lack of early childhood exposure to microbes and infections in the modern era has increased the risk of allergies (Obihara and Bardin, 2008). The hygiene hypothesis was first raised by Strachan who noted that larger numbers of older siblings seemed to be protective against the acquisition of allergic rhinitis (Strachan, 1989). Although some have discounted the hygiene hypothesis (Brooks et al., 2013), there is recent evidence that credits this theory in understanding allergies (Liu, 2015). At a molecular level, T- helper 1 and 2 balance may explain the hygiene hypothesis.

The T-cell mediated immune response is part of the adaptive immune reaction in human beings (Murphy, 2012). When a naïve T-cell encounters an antigen on an antigen presenting cell, it proliferates and differentiates into effector cells known as T-helper (TH), TH1, TH2, TH17, TH22, regulatory T cells and others depending on the exposures, immune milieu and genes (Murphy, 2012, Sompayrac, 2008). These effector cells perform different functions (Murphy, 2012). TH1 is involved in cell mediated immunity and inflammation against intracellular pathogens (Figure B1) (Murphy, 2012, Sompayrac, 2008). TH2 is involved in promoting antibody mediated immunity against parasites (Figure B1) (Sompayrac, 2008, Murphy, 2012). An inappropriate TH1 response causes autoimmunity, and an inappropriate TH2 immune response is associated with an increased risk of allergies (Murphy, 2012).

In atopic individuals when the body encounters an allergen, it activates the TH2 which induces an allergen specific T-cell and B-cell memory response through cytokines, interleukin 4, 5, and 13 (Sompayrac, 2008, Valenta et al., 2015). At this stage sensitisation has occurred, but counter-regulatory mechanisms may prevent the person from exhibiting symptoms on future exposure (Murphy, 2012). If regulatory mechanisms fail, subsequent exposure will lead to symptoms (Murphy, 2012). If that is the case, the allergen cross links with an IgE bound on a

mast cell leading to mast cells releasing granules which results in allergic inflammation (Sompayrac, 2008).

**Figure B1: Illustration of TH1 and TH2 pathways**




*http://www.wellnessalternatives-stl.com/am-i-th1-or-th2-or-th17/*

Reduced exposure to infections and lower microbial diversity of the microbiome in humans leads to a decreased TH1 response and fosters a predominant TH2 type response in the body (Matheson et al., 2010). However, a downregulation of TH1 and upregulation of TH2 does not explain why there has been an increase in autoimmunity and inflammatory bowel diseases which are TH1 mediated with an increase in the prevalence of allergic disorders (Rook and Brunet, 2005). Rook and Brunet, (2005) commented that the balance of TH1 and TH2 may not be as important as the balance between T-effector cells and T-regulatory cells (Rook and Brunet, 2005). Diminished activity of T-regulatory cells may be reason for the increase in both allergic disorders (TH2) and autoimmunity and inflammatory bowel diseases (TH1) (Rook and Brunet, 2005). Decreased exposure to nonvirulent microorganisms such as helminths and lactobacilli leads to decreased activity of T- regulatory cells which are involved in inhibiting allergic disorders (Rook and Brunet, 2005). Therefore these type of organisms have been coined “old friends” and hence the “old friends hypothesis” has been used to explain the mechanism behind the hygiene hypothesis (Rook and Brunet, 2005).

Studies investigating the relationship between immunisation and atopic disorders exist, however, there are few studies that have investigated the relationship between immunisation and food allergy. On one hand, immunisations may increase the risk of allergies through protecting against infections or by exposing the individual to the vaccine adjuvants which may encourage a TH2 pathway (Matheson et al., 2010). On the other hand, exposure to the antigen incorporated in a vaccine may stimulate the TH1 type immune response and therefore, result in immunisation protecting against allergies (Rottem and Shoenfeld, 2004).

In light of this, the objectives of this literature review are as follows;

## Objectives

1. To examine existing evidence for associations between vaccines and food allergies and sensitisation.
2. To examine existing evidence for associations between vaccines and atopy including; asthma, eczema (atopic dermatitis), rhinoconjunctivitis (hay fever).

## Literature Search strategy

Two databases, Medline and Scopus were used to search for articles related to vaccines, food allergies and atopies. The search terms used to conduct the literature search are shown in table B1.

**Table B1: Search terms and returned hits**

| **MEDLINE SEARCH ON PUBMED** | results |
|---|---|
| ((((((((((vaccines) OR vaccines) OR vaccination) OR vaccinations) OR immunization) OR immunizations) OR immunisation) OR immunisations)) OR (((("Vaccination"[Mesh])) OR "Vaccines"[Mesh]))) AND (((((((food allergies) OR food allergy) OR food sensitivity) OR food sensitization) OR food sensitisation) OR food hypersensitivity)) OR "Food Hypersensitivity"[Mesh]) | 1267 |
| (((((((((Vaccination) OR Vaccinations) OR Vaccine) OR Vaccines) OR Immunisation) OR Immunisations) OR Immunization) OR Immunizations)) AND ((((((((((Atopy) OR Atopies) OR atopic) OR atopic dermatitis) OR eczema) OR allergic rhinitis) OR hay fever) OR asthma) OR Rhinoconjunctivitis) OR allergies) OR allergy) AND ("last 5 years"[PDat]) | 3990 |
| **SCOPUS** | |
| ( vaccination OR vaccinations OR vaccine OR vaccines OR immunisation OR immunisations OR immunization OR immunizations ) AND ( food allergies OR food allergy OR food sensitization OR food sensitizations OR food sensitisation OR food sensitisations OR atopy OR atopies OR atopic OR allergies OR allergy OR atopic dermatitis OR eczema OR allergic rhinitis OR hay fever OR rhinoconjunctivitis OR asthma ) AND ( EXCLUDE ( DOCTYPE , "ch" ) OR EXCLUDE ( DOCTYPE , "bk" ) OR EXCLUDE ( DOCTYPE , "ed" ) ) | 2418 |

Following the literature search the articles were then sorted according to the articles that had the search terms in the title and abstract. The titles of the first 100 articles were scanned for each search. If the title indicated that the study looked at the relationship between vaccines and allergies, the abstract was assessed to determine if the article contained the appropriate information. The first 100 were searched because the relevant literature was displayed in the first 50 articles within each search, by the 75$^{th}$ article the papers required for this literature review were no longer being picked up by the search engines. Only English articles that looked at vaccinations that are administered in the public health system in South African to children up until 18 months were considered (OPV, BCG, RV, PCV, DTaP-IPV/Hib, DTaP-IPV-Hib-HBV and measles). Further, only experiments that were carried out in humans were considered when the abstracts were being reviewed. A restriction of articles within the last five years was placed on the second article search carried out on Medline through PubMed due to a high number of articles returned. The search on Scopus excluded book chapters, books and

editorials. In addition, the bibliography of articles were searched to ensure that key articles in the field were included in the literature review. Articles that information was drawn from to write the literature review are summarised in table B2.

**Table B2: Summary of articles included in the literature review**

| Author | Study design | Setting | Population | Vaccine | Allery or atopy | Measure of Association |
|---|---|---|---|---|---|---|
| Aaby 2000 | Cross sectional | Guinea-Bissau | Children 3-14 yrs N = 400 | Documented BCG | Atopy (SPT) | OR 0.19 (0.06- 0.59) |
| | | | | BCG scar only | Atopy (SPT) | OR 0.94 (0.42 to 2.08) |
| Arnoldussen 2011 | Systematic review | 4 databases | 17 articles children aged 17 years or less | BCG | Asthma | OR 0.73 (0.56-0.95 |
| | | | | | Sensitization to common allergens (IgE) | OR 1.31 (1.07-1.60) |
| | | | | | Sensitization to common allergens (SPT) | OR 0.87 (0.67–1.13) |
| | | | | | Eczema | OR 0.84 (0.64-1.09) |
| | | | | | Rhinoconjunctivitis | OR 1.07 (0.89- 1.28) |
| | | | | | Allergy in general | OR 0.84 (0.67-1.05) |
| Bersen 2006 a | Cross sectional | Netherlands | children attending orthodox reformed (Protestant) primary schools 8–12 yr n = 1 872 | DTaP-IPV | Food allergy | OR 1.13 (0.71–1.81) |
| | | | | | Asthma | OR 1.04 (0.76–1.42) |
| | | | | | Hay fever | OR 0.79 (0.55–1.12) |
| | | | | | Eczema | OR 0.87 (0.66–1.14) |
| | | | | | Any atopic disorder | OR 1.00 (0.80–1.24) |
| Bernsen 2006 b | Cross sectional | Netherlands | children attending orthodox reformed (Protestant) primary schools 8–12 yr n = 1 201 | Hib | Food allergy | OR 0.68 (0.38–1.19) |
| | | | | | Asthma | OR 0.89 (0.55–1.43) |
| | | | | | Hay fever | OR 0.94 (0.47–1.90) |
| | | | | | Eczema | OR 1.09 (0.75–1.58) |
| | | | | | Any atopic disorder | OR 1.09 (0.79–1.50) |
| Black, 2000 | RCT | Northern California Kaiser Permanente | 2, 4, 6 and 12 to 15 months 37 868 children Selected outpatient clinic visit categories 3 days from vaccine | PCV | Allergic reactions | P = 0.677 |
| | | | | PCV | asthma, wheezing, shortness of breath, or breath holding | P = 0.522 |

| Author | Study design | Setting | Population | Vaccine | Allery or atopy | Measure of Association |
|---|---|---|---|---|---|---|
| de Andrade 2013 | Cross sectional | Brazil | schoolchildren and adolescents 13- 14 yrs N = 2213 | BCG revaccination | asthma | OR 0.68 (0.37- 1.25) |
| | | | | | allergic rhinitis and/or atopic eczema | OR 1.07 (0.84 -1.36) |
| Dulny 2015 | Cross sectional | Poland | 6-44 year olds N= 18617 | Measles | Symptomatic asthma | OR 0.80 (0.71–0.91) |
| | | | | | Symptomatic allergic rhinitis | OR 1.21 (1.06–1.34) |
| | | | | | Symptoms rhinitis | OR 1.22 (1.11–1.32) |
| Flohr 2012 | Cross sectional | International Study | schoolchildren 8-12 yrs n= 23,901 | BCG vaccination < 1 yr | Wheeze, hay fever, eczema Asthma, common allergens | P>0.05 |
| | | | | BCG vaccination >1 yr | Wheeze, hay fever, eczema Asthma, common allergens | P>0.05 |
| | | | | | Flexural eczema on skin examination | OR 1.82 (1.14–2.91 |
| Floistrup 2006 | Cross-sectional | 5 European countries | Children 5 -13 yrs 4606 from Steiner schools (anthroposophic lifestyle) 2024 from reference schools | MMR Measles Mumps &Rubella | Rhinoconjunctivitis symptoms | OR 1.43 (1.04-1.96) |
| | | | | | Doctor's diagnosis of rhinoconjunctivitis | OR 1.58 (1.05-2.38) |
| | | | | | Overall allergic risk (doctor's diagnosis of rhinoconjunctivitis, and/or asthma, and/or atopic eczema) | OR 0.88 (0.72-1.07) |
| | | | | | Wheezing | OR 0.75 (0.55-1.02) |
| | | | | | Doctor's diagnosis of asthma | OR 0.77 (0.57-1.03) |
| | | | | | Current atopic eczema symptoms | OR 0.89 (0.69-1.16) |
| | | | | | Doctor's diagnosis of atopic eczema | OR 0.81 (0.62-1.06) |
| | | | | | Atopic sensitization | OR 0.91 (0.63-1.31) |
| Gruber 2008 | Cross sectional | Centres in Europe, South Africa and Australia | Infants 1–2 yrs n = 2184 | polio | Food sensitisation | OR 2.60 (1.08–6.25) |
| | | | | hepatitis B | elevated total IgE | OR 1.48 (1.03–2.13) |
| | | | | pneumococci | Elevated total IgE | OR 0.49 (0.27–0.92) |
| | | | | polio | eczema severity | OR 0.66 (0.45–0.97) |
| | | | | pertussis | eczema severity | OR 0.30 (0.10–0.89) |
| | | | | measles | eczema severity | OR 0.63 (0.49–0.80) |

| Author | Study design | Setting | Population | Vaccine | Allery or atopy | Measure of Association |
|---|---|---|---|---|---|---|
| Kiraly 2016 | Cross sectional | Australia | HealthNuts cohort 12 month old infants N = 4 433 | DTaP | food allergy | OR 0.77 (0.36–1.62) |
| | | | | DTaP | atopic sensitization | OR 0.66 (0.35–1.24) |
| Kiraly 2013 | Cross sectional | Guinea–Bissau | low-birthweight infants followed up for 3- 9 yrs | early and delayed BCG | atopy | OR 0.71 (0.34–1.47) |
| | | | | BCG with a scar | atopy | OR 0.42 (0.19–0.94) |
| Klugman, 2003 | rct | South Africa (non HIV + children) | 6, 10, and 14 wks of age 19,922 children cases 19,914 controls Asthma HAD 31 days after receiving vaccine | PCV | Asthma and Hyperactive-airway Disease (59 vaccine recipients and 33 controls) | RR 1.79 p = 0.009 |
| | | | | PCV | Multiple episodes of asthma (22 cases among vaccine recipients, as compared with 12 among controls) | RR 1.83 p = 0.12 |
| | | | | PCV | Children ≥12 months of age Asthma and Hyperactive-airway (42 vs. 22 cases) | RR = 1.91 p = 0.02 |
| Linehan 2014 | Prospective cohort study and Systematic review | Manchester Community Asthma Study MACAS Manchester | Children Follow up ~13-17 yrs n = 1608 | BCG | wheezing | RR 1.05 (0.94-1.19) |
| | | | | | hay fever/ eczema | RR 0.97 (0.89-1.06) |
| | | | | Meta-analysis BCG | Asthma | RR 0.95 (0.89-1.00) |
| | | | | | Sensitization to common allergens (IgE) | OR 1.05 (0.65-1.69) |
| | | | | | Sensitization to common allergens skin prick test (SPT) | OR 0.93 ( 0.83-1.04) |
| | | | | | Eczema | OR 0.85 ( 0.68-1.08) |
| | | | | | Rhinoconjunctivitis | OR 1.06 (0.91-1.23) |
| | | | | | Allergy in general | OR 0.94 (0.83-1.07) |

| Author | Study design | Setting | Population | Vaccine | Allery or atopy | Measure of Association |
|---|---|---|---|---|---|---|
| Matheson 2010 | Prospective cohort | Tasmania | TAHS<br>Adults 44yrs<br>n = 5729 | diphtheria | Food allergy | RR 1.09 (0.81, 1.47) |
| | | | | tetanus | | RR 1.03 (0.78; 1.35) |
| | | | | Pertussis | | RR 1.11 (0.84; 1.47) |
| | | | | Polio | | RR 1.11 (0.80; 1.53) |
| | | | | DTP | | RR 1.02 (0.80; 1.30) |
| | | | | diphtheria, tetanus, Pertussis, Polio, DTP | Eczema, asthma, hay fever | P> 0.05 |
| McKeever 2004 | Cross section | United Kingdom | Children 0- 11 yrs<br>n= 29 238 | DPPT | asthma | HR 10.33 (5.36- 19.91) |
| | | | | DPPT | eczema | HR 7.51 (5.27- 10.70) |
| | | | | MMR | asthma | HR 2.20 (1.50, 3.21) |
| | | | | MMR | eczema | HR 3.50 (2.38, 5.15) |
| Nakajima, 2007 | Retrospective cohort study | Tasmania | Children born 1961<br>N = 8443<br>Follow up<br>at 7, 13 and 30 yrs | Diphtheria | Food allergy by age 7 years | OR1.47 (1.04 to 2.07) |
| | | | | Tetanus | Food allergy by age 7 years | OR 1.26 (0.93 to 1.71) |
| | | | | Pertussis | Food allergy by age 7 years | OR 1.39 (1.01 to 1.91) |
| | | | | Polio | Food allergy by age 7 years | OR 1.44 (1.00 to 2.07) |
| | | | | Diphtheria | Asthma by age 7 years | OR 1.31 1.33 (1.06 to 1.68) |
| | | | | Diphtheria | Eczema by age 7 years | OR 1.53 (1.13 to 2.07) |
| | | | | Tetanus | Eczema by age 7 years | OR 1.53 (1.15 to 2.04) |
| | | | | Pertussis | Eczema by age 7 years | OR 1.51 1.46 (1.10 to 1.93) |
| Park 2015 | Cross sectional | Korean | adults 18-86 yrs<br>n = 200 | BCG scar | 38 unspecified common allergens | P>0.05 |
| | | | | | asthma | OR 0.33 (0.14- 0.77) |

| Author | Study design | Setting | Population | Vaccine | Allery or atopy | Measure of Association |
|---|---|---|---|---|---|---|
| Singh 2013 | Cross sectional study | North India | children 7–14 yrs n = 10,028 | BCG | Sensitisation | OR 0.76 (0.59-0.98) |
| | | | | DTP, Polio, measles | Sensitisation | P>0.05 |
| Timmerman 2015 | Danish birth cohort | Denmark | birth cohort followed up at 5, 13 & 15 yrs | MMR | 5yrs asthma | OR 0.32 (0.10; 1.05) |
| | | | | | 13yr asthma ever | OR 0.16 (0.05; 0.53) |
| | | | | | 13yrs rhinoconjunctivitis ever | OR 0.63 (0.14; 2.71) |
| | | | | | 13yrs eczema ever | OR 0.46 (0.14; 1.52) |
| | | | | | 13yrs any allergen (SPT) | OR 0.47 (0.16; 1.40) |
| Vogt 2014 | Prospective cohort study | Sweden | Adolescents 15 yrs 80 000 vaccinated 98 475 unvaccinated | DTaP/DTP | asthma medication use | OR 0.99 (0.95–1.03) |
| | | | | | asthma anti-inflammatory treatment | OR 0.97 (0.92–1.01) |
| Yemaneberhan 2006 | Cross-sectional household survey | Ethiopia | household survey n = 12 876 | Pertussis | atopic dermatitis | OR 0.60 (0.37-0.99) |
| | | | | measles | | OR 0.79 (0.49–1.28) |
| | | | | diphtheria | | OR 0.72 (0.44–1.18) |
| | | | | BCG | | OR 0.81 (0.51–1.28) |
| | | | | tetanus | | OR 0.98 (0.65–1.47) |
| | | | | polio | | OR 0.76 (0.47–1.25) |

*OR: odds ratio, SPT: skin prick test, wks: weeks yr: year, DTaP-IPV/Hib: Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b, DTaP: Diphtheria–tetanus–acellular pertussis, DTP: Diphtheria tetanus pertussis, BCG: bacillus calmette guerin, PCV: pneumococcal conjugate vaccine, MMR: measles mumps and rubella, Hib: Haemophilus Influenza Type b, IgE: Immunoglobulin E*

## Summary and interpretation of literature

### Immunisation and allergy

#### BCG (Bacillus Calmette–Guerin)

***Negative association: BCG and allergies***

An inverse relationship between the Mycobacterium Tuberculosis Bacillus Calmette–Guerin (BCG) vaccine and atopic diseases has been identified in studies carried out in different parts of the world (Obihara and Bardin, 2008). In Guinea Bissau, 3-14 year old children who had a BCG vaccination scar or documentation confirming a BCG vaccination had a negative association (OR 0.19; 95% CI: 0.06 - 0.59) with sensitisation to house dust mites and cockroach mix (any SPT wheel ≥2) compared to those who hadn't had a BCG vaccination in a sample of 394 (Aaby et al., 2000).

Similarly, an additional study carried out in the same region showed that in 273 children, those who were BCG vaccinated and developed a scar (85%) had a 58% (OR 0.42; 95% CI: 0.19–0.94) decrease in odds of sensitisation (any SPT wheel ≥3mm to egg white, peanuts, cow's milk, house dust mites or cockroach) compared to those who were vaccinated and didn't develop a scar (Kiraly et al., 2013). The same study found that the time of BCG vaccination, early or delayed, was not significantly associated with sensitization to egg white, peanuts, cow's milk, house dust mites or cockroach and symptoms of atopic disease (Kiraly et al., 2013). In the study by Kiraly et al., (2013), BCG vaccination was administered in infancy and the researchers tested atopic symptoms and sensitization outcomes 3- 7 years later (Kiraly et al., 2013). The vaccinated group that didn't develop a scar may have had other factors that led to a larger prevalence of atopy compared to those who developed a scar, thereby confounding the relationship between BCG scar and the negative association with

atopy. Nevertheless, the above Guinea Bissau studies by Aaby et al. (2000) and Kiraly et al. (2013) indicate that BCG may be protective for atopy in African populations.

An inverse relationship between the BCG vaccine and atopic diseases has been identified in studies carried out in Asia. Amongst children between 7-14 years in India, BCG was negatively associated (OR 0.76; 95% CI: 0.59-0.98) with sensitisation to apple, wheat, cow milk, hen's egg, peanut, grass, cockroach, house-dust mite and dog dander (skin prick test wheal $\geq$ 3mm) (Singh et al., 2013). DTaP, Polio and measles vaccinations were not significantly (P>0.05) associated with sensitisation in this cohort (Singh et al., 2013). This study indicates that BCG immunisation may be protective for food and aeroallergen sensitisation in Asian populations.

In Korean adults, between ages 18-86 years, a BCG scar was not significantly (P$\geq$0.05) associated with sensitisation to 38 unspecified common allergens tested through SPTs (Park et al., 2015). However, a BCG scar was found to be inversely associated (OR 0.33; 95% CI: 0.14, 0.77) with asthma confirmed through bronchodilator reversibility or airway hyper responsiveness and symptoms (Park et al., 2015). It is likely that the participants in the Park et al. (2015) study, had received other childhood vaccinations besides BCG. It is possible that BCG and other vaccines may have resulted in the negative association identified. Overall, the studies above provide evidence that BCG may be protective for food and respiratory allergy.

***Positive association: BCG and allergies***

Children who were BCG vaccinated after the first year of life were more likely to experience flexural eczema on their skin compared to those not vaccinated (OR 1.82 95% CI: 1.14–2.91) (Flohr et al., 2012).

### *Insignificant association: BCG and allergies*

Contrastingly, in 2014, a prospective cohort study investigated the effect of BCG vaccination on self-reported wheezing and hay fever and or eczema in adolescents between ages 13- 17 years (Linehan et al., 2014). No significant association was found in this cohort of 1608 individuals (Linehan et al., 2014). The authors noted an association in a subset of the cohort at 7-11 years, showing the effect of BCG maybe temporary (Linehan et al., 2014). This same study included an update to a systematic review and meta-analysis which consisted of 22 non experimental epidemiological studies (Linehan et al., 2014). From the meta-analysis the authors found a (non-significant) lower prevalence of asthma (OR 0.95; 95% CI: 0.89-1), eczema (OR 0.85; 95% CI; 0.68-1.08) and allergy in general (OR 0.94; 95% CI: 0.83-1.07) for those who were BCG vaccinated compared to those who were not (Linehan et al., 2014). The prevalence of rhinoconjunctivitis (OR 1.06; 95% CI, 0.91-1.23) and sensitization to common allergies tested by IgE blood tests (OR 1.05; 95% CI: 0.65-1.69) was higher and statistically insignificant in BCG vaccinated subjects. (Linehan et al., 2014). However the prevalence to common allergies when tested through SPTs was lower and insignificant (OR 0.93; 95% CI: 0.83-1.04) (Linehan et al., 2014). The study highlights the importance of the outcome measure used for diagnosing “allergies” or “atopy” and how this can affect estimates.

A Brazilian study sought to investigate whether a second BCG vaccination administered 7- 10 years after the neonatal one would be associated with self-reported asthma (de Andrade et al., 2013). A sample of 2213 were BCG vaccinated 7- 10 years after a neonatal vaccine and followed up for 3- 8 years to assess asthma through the ISAAC questionnaire (de Andrade et al., 2013). The study found no significant association between BCG revaccination and disease outcomes (de Andrade et al., 2013). Different results, negative or lack of association, from

different studies looking at a similar association indicate the importance of carrying out further research that will add towards reaching definitive conclusions.

**DTaP/DTP (Diphtheria, Tetanus and Pertussis) & Polio**

*Positive association: DTP and allergies*

1501 individuals from Tasmania participated in a retrospective cohort study investigating the association between several childhood vaccines and self-reported atopic diseases and self-reported food allergies (Nakajima et al., 2007). The individuals consisted of children born in 1961 and the study followed up the participants at 7, 13 and 30 years of age (Nakajima et al., 2007). The study found that the adjusted association between vaccination and food allergies at age 7 was positive for both diphtheria (OR 1.47; 95%CI: 1.04-2.07) and whole pertussis (OR 1.39; 95%CI: 1.01 -1.91) vaccinations (Nakajima et al., 2007). The association for eczema was positive for those vaccinated with diphtheria (OR 1.53; 95%CI: 1.13 to 2.07), tetanus (OR 1.53; 95%CI: 1.15-2.04) and whole pertussis (OR 1.46; 95%CI: 1.10-1.93) (Nakajima et al., 2007).The relative odds ratio for asthma and diphtheria (OR 1.33; 95%CI: 1.06-1.68) vaccinated individuals was positive 1.33 (95%CI: 1.06-1.68) (Nakajima et al., 2007). These associations became insignificant after 13 years of follow up except for the association between eczema and whole pertussis vaccination, where vaccinated subjects now had a lower odds ratio for eczema (OR 0.57; 95%CI: 0.35-0.93) (Nakajima et al., 2007). Therefore, the age at which allergies are assessed in relation to vaccines is important and different effects may be dominant at different ages.

A British study found that the hazards of incident diagnosed asthma (HR 10.33; 95%CI: 5.36-19.91) and incident diagnosed eczema (HR 7.51; 95%CI: 5.27- 10.70) in a cohort of 29 238 children was greater for those who received diphtheria, polio, whole pertussis, and tetanus

(DPPT) vaccination, when controlling for GP visits (McKeever et al., 2004). A strength of this study is the large sample size, 29 238 providing high power, although the associations may have been identified with a smaller study, but with less precision.

***Negative association:*** *DTP + Polio and allergies*

A negative association between DTP and atopy has been identified in other parts of the world. Gruber et al., (2008) carried out an international study that looked at the association between 13 childhood vaccines (diphtheria, tetanus, whole &acellular pertussis, polio, haemophilus influenza Type B, hepatitis B, mumps, measles, rubella, varicella, BCG, meningococci and pneumococci) and food allergens in infants between 1-2 years (Gruber et al., 2008). Polio was found to have a positive increased effect on food sensitisation (IgE $\geq$ 0.35kU/L to one or more; milk, egg and peanut) (OR 2.60 95% CI: 1.08–6.25) (Gruber et al., 2008).

Lastly, an Ethiopian study was carried out, to investigate the effect of lifestyle factors on atopic dermatitis (Yemaneberhan et al., 2004). The study, which consisted of 12 876 individuals, reported whopping cough vaccine to be negatively associated with atopic dermatitis (OR 0.60; 95% CI: 0.37-0.99) (Yemaneberhan et al., 2004). However, BCG, measles, diphtheria, small pox, mumps tetanus and polio vaccines were not significantly associated with self-reported atopic dermatitis and SPT $\geq$3mm for dust mites, aspergillus and mixed threshing (Yemaneberhan et al., 2004). This study was carried out in Africa and gives insight into possible associations that may be found in a South African cohort.

***Insignificant association:*** *DTP or Polio and allergies*

Although some studies have found positive and negative associations between DTP and atopy or food allergies, other studies have found no significant association between the variables.

A Swedish prospective cohort study, found no significant association between DTP (whole and acellular pertussis) immunisation and asthma medication use (OR 0.99 95%CI: 0.95–1.03) and asthma anti-inflammatory treatment (OR 0.97 95%CI: 0.92–1.01) (Vogt et al., 2014). This study investigated asthma in adolescent participants who were exposed to DTP as infants (Vogt et al., 2014). It is possible that there may have been participants who were asthmatic but not taking medication and if this was related to vaccine exposure this would have resulted in misclassification and incorrect estimates (Vogt et al., 2014).

In another study, of cross sectional design, no significant association was identified between those vaccinated with diphtheria, tetanus, acellular pertussis and poliomyelitis (DTaP-IPV) vaccination during infancy and questionnaire obtained data on food allergies (OR: 1.13 95% CI: 0.71–1.81) or asthma (OR: 1.04 95% CI: 0.76–1.42), eczema (OR: 0.87 95% CI:0.66–1.14) , hay fever (OR: 0.79 95% CI: 0.55–1.120) and any atopic disorder (OR: 1.00 95% CI: 0.80–1.24) in 8- 12 year olds in the Netherlands (Bernsen et al., 2006a). Although a questionnaire was used to collect data on the exposure and outcome variables, the authors did validate a subset of the sample with IgE blood tests, improving the validity of the findings.

Similarly, Matheson et al. (2012) carried out a study looking at the association between childhood immunisation and any occurrence of self-reported food allergy and atopic disease. The authors investigated childhood immunisation status for diphtheria, tetanus, pertussis, poliomyelitis and small pox (Matheson et al., 2010). The adjusted risk ratios for food allergy when exposed to the following vaccines were insignificant; diphtheria 1.09 (95%CI: 0.81-1.47) tetanus 1.03 (95%CI: 0.78-1.35), whole pertussis 1.11 (95%CI: 0.84-1.47), Polio 1.11 (95%CI:

0.80-1.53) and DTP 1.02 (95%CI: 0.80-1.30) (Matheson et al., 2010). Furthermore, there were no significant associations between any of the vaccines and asthma in the last 12 months or ever having had food eczema or hay fever (Matheson et al., 2010). This study was carried out on a sample size greater than 5550 individuals increasing the power of the study to find a significant association. A questionnaire completed by parents in 1968 before the individuals in cohort began school was used to determine exposure to immunisation and a questionnaire administered in 2004 was used to determine atopy and food allergy outcomes (Matheson et al., 2010). Therefore, recall bias may have occurred in this study. Secondly, the questionnaire completed in 2004 was self-administered by participants who were not specifically in the medical field (Matheson et al., 2010). Consequently, there is a possibility that outcomes may have been completed inaccurately. An advantage of this cohort study is temporality. Exposure was assessed and the outcome was measured over time thereafter.

The Australian Healthnuts study is the only study that has investigated the relationship between DTaP vaccination and challenge proven food allergies in infants (Kiraly et al., 2016). The cross sectional study revealed that there was no association between DTaP vaccination and food allergies (Kiraly et al., 2016). However, the study did demonstrate that DTaP administered with a month delay was negatively associated with self-reported eczema (OR 0.57; 0.34- 0.97, p=0.04) in comparison to when the vaccine was administered at the standard time (Kiraly et al., 2016). Although this finding may have been due to reverse causation, it does raise the possibility that the timing of vaccinations may influence the association with atopy. Inconclusive results on the association between DTP and polio vaccinations and atopy warrants further research to be carried out.

Part B Literature Review

**PCV (pneumococcal conjugate vaccine)**

***Positive association: PCV and allergies***

The pneumococcal conjugate vaccine (PCV) is positively associated (RR 1.79; p = 0.009) with asthma and hyperactive-airways in South African children 31 days after receiving the vaccination (Klugman et al., 2003). Similarly, PCV is positively associated (RR = 1.91; p = 0.02) with asthma and hyperactive airways in children greater than 12 months of age (Klugman et al., 2003). However, no significant association was observed for multiple episodes of asthma and PCV in these children (RR 1.83; p = 0.12) (Klugman et al., 2003). In California, PCV was found to be non-significantly associated with allergic reactions and asthma (P>0.05) (Black et al., 2000).

**MMR (Measles Mumps and Rubella)**

***Positive association:*** *MMR and allergies*

The MMR vaccine has been found to be positively associated with atopy in different parts of Europe. In Poland, Measles was found to be positively associated with both self-reported symptomatic rhinitis (OR 1.22; 95%CI: 1.11–1.32) and allergic rhinitis (OR 1.21; 95%CI: 1.06–1.34) in participants who ranged from 6 and 44 years of age in a cross sectional study (Dulny et al., 2015). However, these associations didn't hold for doctor diagnosed or SPT diagnosed atopy (Dulny et al., 2015). In another MMR study, in order to find children who had not received an MMR vaccination, children from Steiner schools who were unlikely to be vaccinated were participants in the investigation (Floistrup et al., 2006). The study showed that MMR was positively associated with rhinoconjunctivitis symptoms (OR: 1.43; 95%CI: 1.04-1.96) and doctor's diagnosed rhinoconjunctivitis (OR: 1.58 95%CI: 1.05-2.38) (Floistrup et al., 2006). However, no significant association was found between MMR and self-reported wheezing (OR: 0.75 95%CI: 0.55-1.02), self-reported eczema (OR: 0.89 95%CI: 0.69-1.16),

doctor diagnosed eczema (OR: 0.81 95%CI: 0.62-1.06) and asthma (OR: 0.77 95%CI: 0.57-1.03) and atopic sensitisation (IgE level ≥ 0.35 kU/L.) (OR: 0.91 95%CI: 0.63-1.31) (Floistrup et al., 2006). A weakness of this study is the possibility for confounding as Steiner children may have multiple other differing exposures. Similarly, McKeever et al (2004), found that: children with MMR vaccination had a significantly increased risk in clinically diagnosed eczema (hazard ratio 3.50; 95%CI: 2.38- 5.15) and asthma (hazard ratio 2.20; 95%CI: 1.50- 3.21) compared to those who were not vaccinated (McKeever et al., 2004).

***Negative association:*** *MMR and allergies*

Other studies have shown MMR to be negatively associated with atopy. The study by Dulny et al., (2015), reported a negative association between measles vaccination and self-reported asthma (OR 0.80, 95%CI: 0.71–0.91) and no significant association regarding doctor diagnosed or SPT diagnosed asthma (Dulny et al., 2015). Similarly, in a Faroe islands birth cohort, the measles mumps and rubella (MMR) vaccine was found to be significantly associated with a reduced odds of 0.16 (95%CI: 0.05-0.53) of asthma 13 year old children (Timmermann et al., 2015). MMR is administered at 12 months and 12 years in Denmark and the birth cohort was followed up at 5, 13 and 15 years (Timmermann et al., 2015). At 5 years the association with asthma was insignificant. This may point to the transient nature of a protective association. The follow up included a clinical examination, a health questionnaire completed by the mother and medical history check and an interview (Timmermann et al., 2015). The use of a range of methods for assessing atopy outcomes in this study makes the results robust.

**Hib (Haemophilus Influenza Type b)**

***Insignificant association:*** *Hib and allergies*

A cross sectional study carried out in the Netherlands found that there were no significant association between Haemophilus Influenza Type b (Hib) vaccination and patient-reported food allergies (OR 0.68 95% CI: 0.38–1.19) or patient reported asthma (OR 0.89 95% CI: 0.55–1.43), hay fever (OR 0.94 95% CI: 0.47–1.90) and eczema (OR 1.09 95% CI: 0.75–1.58) in in 1201 participants between 8-12 years of age (Bernsen et al., 2006b).

## Conclusion

### Associations at different ages

The articles above indicate that different studies carried out in different parts of the world result in different types of association between vaccines and food allergies and atopic diseases. Furthermore, the association and strength of association may differ depending on the age that the association is investigated. For example, the study by Nakajima et al (2007), showed that pertussis vaccination was positively associated (OR 1.46; 95%CI: 1.10-1.93) with eczema with 7 year olds and then negatively associated (OR 0.57; 95%CI: 0.35 to 0.93) with eczema with 13 year olds (Nakajima et al., 2007). Similarly, the study by Linehan et al., (2014) and Timmermann et al., (2015) displays this transient nature. Further, some studies investigated the association between vaccinations and atopic diseases in a wide age range of participants. Examples of these studies include the studies looking at the relationship between BCG and atopy in 3-14 year olds (Aaby et al., 2000) olds and 7-14 year olds (Singh et al., 2013) or 18- 86 year olds (Park et al., 2015). If the association is sensitive to age then the effect of vaccines on atopy may not be seen at all ages.

### Measuring outcome

Studies looking at the same vaccination with different results may have been influenced by the way the outcome was assessed. If the outcome was assessed through a questionnaire as

opposed to physical examination or food challenge testing then recall bias might have influenced the estimates. Food allergy has been found to be overestimated in self-reported instances (Rona et al., 2007). Further, some physical examinations involved physical inspection, food challenge testing, SPTs or IgE blood measures. Depending on the robustness of the measure used to estimate the outcome this would affect the validity of the associations found. In addition, exposure to vaccines, was determined in different ways and might have influenced the validity of the associations. Immunisation was assessed through recall, checking medical records or assessing a scar. Using medical records and scars is more reliable than recall. Finally, it is possible that vaccinations may affect different aspects of atopy differently. Moreover, the same vaccine, such as Pertussis, constituted differently, whole or acellular, may affect atopy differently.

**Effect size**

It is important to determine whether there is a negative or positive effect of immunisation on food allergies or atopic diseases, and additionally the effect size of the associations. Many of the significant associations found in the articles described above are small. The associations that indicate a positive association for atopy or food allergies in the articles above have relative odds ratios which are less than 2 and greater than 0.3 for negative effects. In addition, the precision of the effects is important. The smaller a sample size the less precise the estimate.

The articles above indicate that vaccinations may be positively or negatively associated with food allergies. Investigating this phenomenon in a South African cohort will provide insight into modifiable factors that influence food allergy in South African children.

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# PART C

## Manuscript

# Part C: Manuscript

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**Title page**

# THE RELATIONSHIP BETWEEN IMMUNISATION AND FOOD ALLERGY AND SENSITISATION IN SOUTH AFRICAN CHILDREN

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*Key words*: *Immunisation, Vaccination, Food Allergy, Food Sensitisation, South Africa, Children*

| Abbreviations | |
|---|---|
| HREC | Human Research Ethics Council |
| OR | Odds ratio |
| REF | Reference |
| SPT | Skin Prick Test |
| Vaccinations | |
| BCG | Bacillus Calmette Guérin |
| DTaP-IPV/Hib | Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b |
| HBV | Hepatitis B Vaccine |
| MV | Measles vaccine |
| OPV | Oral Polio Vaccine |
| PCV | Pneumococcal conjugate vaccine |
| RV | Rotavirus vaccine |

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**Abstract**

*Background*

The prevalence of food allergy is higher in children than adults and is increasing. The effect of vaccinations on prevalence of food allergy and food sensitisation is not clear.

*Objective*

To determine if a relationship exists between vaccinations and food allergy and food sensitisation in children in the first 18 months of life who live in urban and rural South African communities.

*Methods*

A food allergy and sensitisation survey was conducted in unselected children between 12 and 36 months of age in urban Cape Town and the Mqanduli district of the Eastern Cape. Food sensitisation and allergy were determined through skin prick tests (SPT) and oral food challenges respectively. We examined the association between self-reported immunisation status and food sensitisation and food allergy in children using multivariate logistic regression. Children less than 18 months were excluded from analysis for vaccines offered at 18 months.

*Results*

Among 708 urban and 400 rural children, the numbers of participants positive for food sensitisation and allergy, eczema, hay fever and asthma were significantly greater in the urban sample compared to the rural sample ($p<0.05$). Further, in 708 urban children, those who had a BCG vaccine at birth were 0.05 (OR 0.05; 95% CI: 0.004- 0.6) times less likely to have an SPT $\geq$ 7mm. There were no other significant associations between childhood vaccinations and food sensitization at SPT $\geq$1mm, $\geq$3mm and $\geq$7mm. There was no significant association between vaccinations and food allergy.

*Conclusion*

In conclusion, there was very little evidence of an association between BCG vaccination in children and food allergic sensitisation or food allergy. However, in a small subgroup, there was evidence in an association between BCG and SPT ≥7 mm.

**Key Words**

*Immunisation, Vaccination, Food Allergy, Food Sensitisation, South Africa, Children*

## Introduction

It is estimated that 1-10% of people suffer from a food allergy worldwide (1). The prevalence of some food allergies is higher in children compared to adults. A meta-analysis found that children less than a year old had cow's milk and egg allergy prevalence of 2% and 0.71% respectively compared to 0.61% and 0.23% in adults (2). Food allergy was determined through self-reporting, OFC, SPT or specific IgE testing (2). Further, the prevalence of food allergies has been increasing over time (3, 4). The prevalence of "food allergy" as a discharge diagnosis in hospitalised patients, in the USA, was found to increase significantly ($p < 0.001$) over time from periods 1998 and 2000 to 2001 and 2003 to 2004 and 2006 (5).

The prevalence of food allergies amongst children in South Africa is not well described. One study reported food allergy prevalence amongst children between 6 months and 10 years with atopic dermatitis, a risk factor for food allergies, to be 40% through oral food challenge (OFC) tests (6). Recently, results were published indicating a prevalence of food sensitization (indicated by a skin prick test (SPT) $\geq$1mm) of 12.3% and food allergy of 2.5% amongst 512 unselected urban Cape Town children between 12 and 36 months of age (7). The study reported a prevalence of food allergies (confirmed through an oral food allergy test) to be 1.8%, 0.2%, 1.2% and 0.2% for egg, cow's milk, peanut, and fish respectively (7).

In line with the hygiene hypothesis (8), vaccinations may contribute towards reduced exposure to infections leading to a predominant allergen specific response in the body (9) or, exposure to the virus or microbe in the vaccine may decrease the risk for allergy (10). In Guinea Bissau, children who had a Bacillus Calmette Guérin (BCG) vaccination scar were less

likely to have sensitisation (any SPT wheel ≥ 3mm to egg white, peanuts, cow's milk, house dust mites or cockroach) compared to those who were vaccinated and didn't develop a scar (OR 0.42; 95% CI: 0.19 - 0.94) (11). Similarly, In India, BCG was protective for sensitisation to apple, wheat, cow milk, hen's egg, peanut, grass, cockroach, house-dust mite and dog dander collectively (OR 0.76; 95% CI: 0.59-0.98) (SPT wheel ≥ 3mm) (12). In contrast the Polio vaccine, was found to increase the risk for food sensitisation in children between 1 and 2 years of age (13). Yet other vaccines, Diphtheria–tetanus–pertussis (DTP) and Haemophilus Influenza Type b (Hib), were found to not be associated with food allergy in different parts of the world (9, 14-16).

There have been no previous studies looking at the association between food sensitisation and food allergy in South Africa. Determining if associations between vaccinations and food sensitisation and allergy exist will provide insight into the factors associated with food allergy. As a part of the South African Food sensitisation and Food Allergy (SAFFA) study, the aim of this study is to determine if a relationship exists between vaccinations and food allergy and food sensitisation in children in the first 18 months of life who live in urban Cape Town and in rural Mqanduli in the Eastern Cape.

## Methods

Secondary data analysis of an observational cross sectional study was carried out to determine whether an association between vaccinations and food sensitisation and allergy existed. Participant data was attained from the database of the SAFFA study. 1739 eligible children, 12-36 months of age from registered crèches in metropolitan Cape Town and from communities surrounding health centres in Mqanduli, were recruited for the SAFFA study.

Parents and guardians of eligible participants were invited to participate in the study and provided with information pertaining to the study and thereafter, informed consent was obtained. Demographic data and other information pertaining to participant's background was acquired through administering a questionnaire to parents and guardians.

### Vaccine measures

The vaccination schedule during the time of data gathering is described in Table C1.

**Table C1: South Africa vaccine schedule at time of study**

| Time | Vaccination | Time | Vaccination |
|---|---|---|---|
| Birth | BCG | 14 weeks | RV(2) |
| | OPV (0) | | DTaPIPV/HiB(3) |
| 6 weeks | OPV (1) | | HBV(3) |
| | RV (1) | | PCV7 or 13 (2) |
| | DTaPIPV/HiB (1) | 9 months | Measles vaccine |
| | HBV (1) | | PCV 7 or 13 (3) |
| | PCV7 or 13(1) | 18 months | DTaPIPV/HiB (4) |
| 10 weeks | DTaPIPV/HiB (2) | | Measles Vaccine |
| | HBV (2) | | |

Participants in this study were a minimum of 12 months and a maximum of 36 months, therefore the sample number used to investigate an association with the fourth DTaP-IPV/Hib vaccine and measles vaccine and study outcomes is smaller than the sample number used to investigate the associations of the other 15 vaccines. Vaccine status was ascertained through questionnaire and confirmed through immunisation records if they were available.

### Allergy outcome measures

Each child was tested for food sensitization by means of a skin prick test to the following foods; hen's egg, cow's milk, soy, wheat, fish, peanut and hazelnut. A participant was classified as food sensitised if the result of the SPT was greater than 1mm after subtracting

the size of reaction to the negative controls, and categorised using thresholds greater than 1mm or 3mm or 7mm (8). If a participant was found to have a SPT result greater than 1mm and was not ingesting and "tolerant" to a full age-appropriate portion, they were invited to attend an oral food challenge test which was carried out for the particular food they were sensitised to. A participant was categorised as food allergic if found oral food challenge positive to at least one of the foods they were tested for. Self-reported information on asthma, hay fever and eczema was collected using the following questions; **asthma**- "*has your child ever had symptoms of asthma without having a cold or chest infection? e.g. wheeze, persistent cough at night or when exercising, shortness of breath*", **hay fever**- "*Has your child ever had symptoms of hay fever? e.g. itchy runny eyes, itchy runny nose, blocked nose, frequent sneezing without having a "cold" or upper respiratory tract infection?*" **Eczema**- "*Has your child ever had symptoms of eczema? e.g. an itchy rash especially in the folds of the elbows, behinds the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes"* Appendix 4. These questions are derived from ISAAC studies and have been used in local studies (17).

### Statistical analysis

Participant demographics and comparison of the pattern of immunisation coverage in the rural and urban sites was done using descriptive statistics, chi squared test and wilcoxon rank sum test. We used chi squared tests and logistic regression to investigate univariate associations between immunisation and food allergy and food sensitisation and between immunisation and self-reported asthma, hay fever and eczema. As coverage of many vaccines was 100% in rural areas, we were unable to assess the association between these vaccines and the different outcomes for this area, hence we conducted the analysis separately for urban and rural areas. Similarly, since very few children in the rural area had food allergy, we

did not examine associations between vaccines and food allergy for rural children. Multivariate logistic regression was used to investigate associations between vaccines and the different outcomes adjusting *a priori* for potential confounders of age at enrolment, gender, breast feeding, number of older children in the house and clinically diagnosed eczema. A 95% confidence interval was used to determine significance. All statistical analyses were conducted using Stata 12 (18)

### Ethics

Ethical clearance to carry out this study was granted by the Human Research Ethics Committee from the University of Cape Town, Cape Town, South Africa (HREC REF: 121/2016) Appendix 3.

## Results

Of 1739 eligible children recruited into the study, 1160 responded to the invitation to participate in the study and 1114 actually participated in the study. There were, 1 108 participants who completed the questionnaire and SPT tests; 708 were from urban Cape Town and 400 from rural Mqanduli in the Eastern Cape (Figure C1). Immunisation coverage in the rural participants is greater than that of the urban participants up until vaccines offered from 9 month onwards, however, none of these difference are significantly different ($P \geq 0.05$) (Table C2). The proportion of food sensitised and food allergic participants is significantly greater in the urban sample compared to the rural sample ($p<0.05$) (Table C2). Similarly, atopy outcomes are significantly higher in the urban group compared to the rural group ($p<0.05$) (Table C2). The age at enrolment and the number of older siblings is higher in the urban group ($p<0.05$) (Table C2).

**Figure C1: Diagram illustrating participant flow used in analysis**

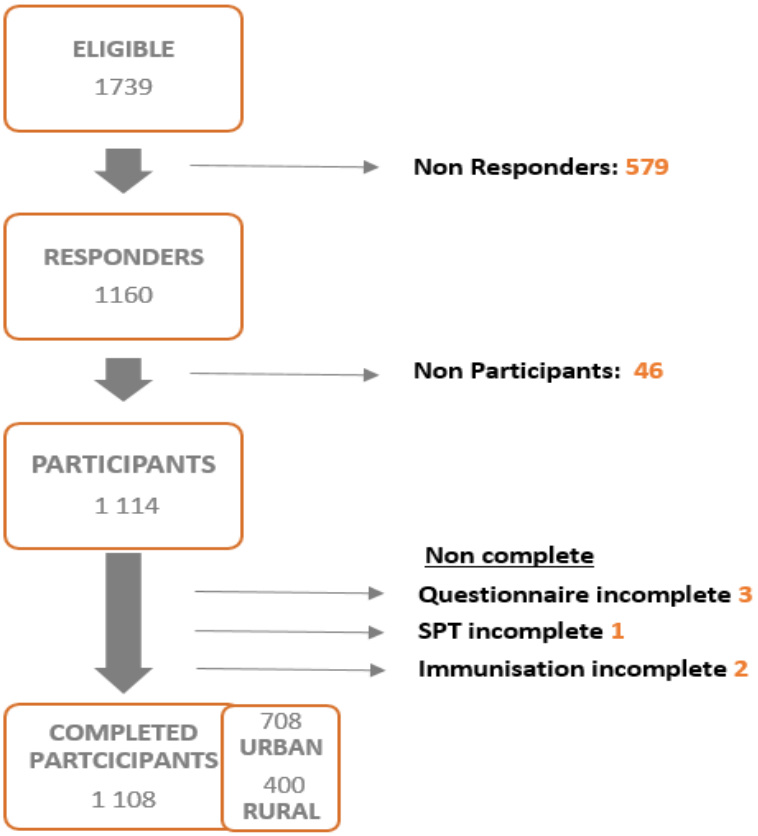

**Table C2: Characteristics of study population**

| Characteristics | % Urban (n) Total n =708 | % Rural (n) Total n =400 | P value |
|---|---|---|---|
| **Immunization status** | **Positive** | **Positive** | |
| Birth | | | |
| BCG | 99.6 (705) | 100 | 0.192 |
| OPV(0) | 99.2 (702) | 100 | 0.065 |
| 6 weeks | | | |
| OPV(1) | 99.4 (704) | 99.7 (399) | 0.452 |
| RV (1) | 99.7 (706) | 100 | 0.287 |
| DTaP-IPV/Hib (1) | 99.7 (706) | 100 | 0.287 |
| HBV(1) | 99.7 (706) | 100 | 0.287 |
| PCV(1) | 99.7 (706) | 100 | 0.287 |
| 10 weeks | | | |
| DTaP-IPV/Hib (2) | 99.6 (705) | 100 | 0.192 |
| HBV(2) | 99.6 (705) | 100 | 0.192 |
| 14 weeks | | | |
| RV (2) | 99.3 (703) | 100 | 0.092 |
| DTaP-IPV/Hib (3) | 99.4 (704) | 100 | 0.132 |
| HBV(3) | 99.6 (705) | 100 | 0.192 |
| PCV(2) | 99.6 (705) | 99.8 (399) | 0.643 |
| 9 months | | | |
| MV(1) | 98.5 (697) | 97.3 (389) | 0.170 |
| PCV(3) | 97.9 (693) | 96.3 (385) | 0.108 |
| 18 months | | | |
| DTaP-IPV/Hib (4) | 91.4 (552) *# n= 604* | 89 (250) *# n= 281* | 0.250 |
| MV2 | 91.4 (552) *# n= 604* | 89 (250) *# n= 281* | 0.250 |
| **Food sensitisation** | **Positive** | **Positive** | |
| SPT ≥ 1mm * | 13.4 (94) | 5.0 (18) | 0.000 |
| SPT ≥ 3mm * | 9.8 (69) | 3.0 (12) | 0.000 |
| SPT ≥ 7mm * | 4.2 (30) | 2.0 (8) | 0.049 |
| **Food allergy** | **Positive** | **Positive** | |
| Oral food challenge * | 4.0 (28) | 1.0 (4) | 0.005 |
| **Atopy** | **Positive** | **Positive** | |
| Self-reported asthma * | 11.3 (80) | 1.0 (4) | 0.000 |
| Self-reported hay fever * | 26.6 (188) | 3.3 (13) | 0.000 |
| Self-reported eczema * | 24.5 (173) | 2.0 (8) | 0.000 |
| **Confounders** | **Positive** | **Positive** | |
| Breast feeding | 87.7 | 88.5 | 0.698 |
| Clinically diagnosed Eczema * | 12.6 | 0.8 | 0.000 |
| Gender | F= 47.0% | F= 43.5% | 0.318 |
| Median age at enrolment (months) * | 27 IQR = 21 - 32 | 21 IQR = 17- 28 | 0.000 |
| Median number of older siblings * | 1 IQR = 0- 1 | 2 IQR = 1-3 | 0.000 |

*SPT: skin prick test, DTaP-IPV/Hib: Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b, BCG: bacillus calmette guerin, PCV: pneumococcal conjugate vaccine, OPV: oral polio vaccine, MV: measles vaccine, HBV: hepatitis B vaccine*
*#The total number of these participants is less than the total 708 for urban and 400 for rural participants due to some participants being less than 18 months of age and not having received the DTaP-IPV/Hib (4) and mv vaccine yet.*
**Significant different between urban and rural for this variable (P<0.05)*

Table C3 shows the associations between vaccinations at birth, 9 months and 18 months and SPT greater than 1mm, 3mm and 7mm. There is a significant protective association between BCG offered at birth and SPT ≥ 7mm (adjusted OR: 0.05 95% CI: 0.004 - 0.6) in the urban sample (Table C2). There are no other significant associations between childhood vaccinations offered up until 18 months and food sensitization as indicated by SPT greater than 1mm, 3mm and 7mm (Table C3 and C4).

**Table C3: Associations between immunisation status and SPT ≥ 1mm, ≥ 3mm and ≥ 7mm in urban and rural participants**

| **Outcome** | | **Urban** | | **Rural** | |
|---|---|---|---|---|---|
| **SPT ≥ 1mm** | **Vaccine** | ***adjusted OR** | **95%CI** | ***adjusted OR** | **95%CI** |
| Birth | BCG | 0.2 | 0.02- 2.7 | | |
| | OPV(0) | 0.3 | 0.1- 1.5 | | |
| 9 months | MV(1) | 1.8 | 0.2- 14.9 | | |
| | PCV(3) | 1.1 | 0.2- 5.0 | 0.6 | 0.1- 5.4 |
| 18 months | DTaP-IPV/Hib (4) | 0.9 | 0.4 - 2.4 | 1.4 | 0.2- 12.0 |
| | MV2 | 0.9 | 0.4 - 2.4 | 1.4 | 0.2- 11.7 |
| **SPT ≥ 3mm** | | | | | |
| Birth | BCG | 0.2 | 0.02- 2.7 | | |
| | OPV(0) | 0.3 | 0.1- 1.5 | | |
| 9 months | MV(1) | 1.8 | 0.2- 14.9 | | |
| | PCV(3) | 1.1 | 0.2- 5.0 | | |
| 18 months | DTaP-IPV/Hib (4) | 0.8 | 0.3- 2.3 | | |
| | MV2 | 0.8 | 0.3- 2.3 | | |
| **SPT ≥ 7mm** | | | | | |
| Birth | BCG | **0.05** | **0.004- 0.6** | | |
| | OPV(0) | 0.2 | 0.02- 1.6 | | |
| 9 months | MV(1) | 0.5 | 0.1- 4.6 | | |
| | PCV(3) | 0.7 | 0.1- 5.7 | | |
| 18 months | DTaP-IPV/Hib (4) | 0.6 | 0.1- 3.1 | | |
| | MV2 | 0.6 | 0.1- 3.1 | | |

*SPT: skin prick test, DTaP-IPV/Hib: Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b, BCG: bacillus calmette guerin, PCV: pneumococcal conjugate vaccine, OPV: oral polio vaccine, MV: measles vaccine,*

*OR: Odds ratio,*
**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema.*
*Where there is a blank in the rural column, there was no difference between exposed and non-exposed in this cohort. Therefore, no odds ratio was calculated.*

**Table C4: Association between immunisation status and food allergy in the urban participants**

| Outcome | Vaccine | OR | 95% CI | *adjusted OR | 95%CI |
|---|---|---|---|---|---|
| **Food allergy** | | | | | |
| Birth | OPV(0) | 0.2 | 0.02- 1.8 | 0.1 | 0.02- 1.3 |
| 9 months | PCV(3) | 0.6 | 0.1 – 4.5 | 0.6 | 0.1- 5.0 |
| 18 months | DTaP-IPV/Hib (4) | 0.9 | 0.2 – 4.1 | 1.1 | 0.2- 5.4 |
| | MV2 | 0.9 | 0.2 – 4.1 | 1.1 | 0.2- 5.3 |

*DTaP-IPV/Hib: Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b, PCV: pneumococcal conjugate vaccine, OPV: oral polio vaccine, MV: measles vaccine, OR: Odds ratio*
**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema*

No significant associations are identified between vaccines and food allergy determined through oral food challenges in the urban cohort (Table C4). For atopy outcomes; asthma, hay fever and eczema were investigated, and there are no significant association between these outcomes and vaccine exposure as shown in table C5 and Appendix 8 and 10.

**Table C5: Association between immunisation status and self-reported eczema, asthma and hay fever for urban and rural participants**

| Outcome | | Urban | | Rural | |
|---|---|---|---|---|---|
| **Self-reported asthma** | **Vaccine** | ***adjusted OR** | **95%CI** | ***adjusted OR** | **95%CI** |
| Birth | OPV(0) | 0.5 | 0.1- 4.6 | | |
| 6 weeks | OPV(1) | 0.3 | 0.03- 3.1 | | |
| 14 weeks | DTaP-IPV/Hib (3) | 0.3 | 0.03- 2.5 | | |
| 18 months | DTaP-IPV/Hib (4) | 0.7 | 0.3- 1.7 | | |
| | MV2 | 0.6 | 0.2- 1.4 | | |
| **Self-reported Hay fever** | | | | | |
| Birth | OPV(0) | 0.7 | 0.1- 3.7 | | |
| 6 weeks | OPV(1) | 0.3 | 0.1- 2.4 | | |

| | | | | | |
|---|---|---|---|---|---|
| 14 weeks | DTaP-IPV/Hib (3) | 0.9 | 0.1 – 8.8 | | |
| 9 months | MV(1) | 1.0 | 0.3 – 4.0 | 0.4 | 0.1- 3.5 |
| | PCV(3) | 1.0 | 0.3- 3.3 | 0.5 | 0.1- 4.0 |
| 18 months | DTaP-IPV/Hib (4) | 0.6 | 0.3 - 1.1 | 0.9 | 0.1- 8.1 |
| | MV2 | 0.7 | 0.4- 1.3 | 0.8 | 0.1- 7.7 |
| **Self-reported eczema** | | | | | |
| Birth | OPV(0) | 0.6 | 0.1- 3.4 | | |
| 6 weeks | OPV(1) | 1.0 | 0.1- 10 | | |
| 14 weeks | RV (2) | 1.4 | 0.2- 13 | | |
| | DTaP-IPV/Hib (3) | 1.0 | 0.1- 10 | | |
| | HBV(3) | 0.7 | 0.1- 7.7 | | |
| 9 months | MV(1) | 0.8 | 0.2 - 3.1 | 0.2 | 0.03- 2.4 |
| | PCV(3) | 0.9 | 0.3 - 2.8 | 0.3 | 0.03- 2.9 |
| 18 months | DTaP-IPV/Hib (4) | 1.2 | 0.6 - 2.6 | 0.2 | 0.02- 2.4 |
| | MV2 | 1.4 | 0.7- 3.1 | 0.3 | 0.02- 2.8 |

*DTaP-IPV/Hib: Diphtheria–tetanus–acellular pertussis / Haemophilus Influenza Type b, PCV: pneumococcal conjugate vaccine, OPV: oral polio vaccine, MV: measles vaccine, HBV: hepatitis B vaccine, OR: Odds ratio,*
**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema. Self-reported eczema odds ratio adjusted for age at enrolment, gender, breast fed and number of older children in the house.*
*Where there is a blank in the rural column, there was no difference between exposed and non-exposed in this cohort. Therefore, no odds ratio was calculated.*

## Discussion

This is the first time a study of this nature, looking at the association between vaccines and food allergy and food sensitisation, has been carried out in South Africa. In this study, those children who had a BCG vaccine at birth were found to be 0.05 (95% CI: 0.004 - 0.6) times less likely to have an SPT ≥ 7mm when confounders were adjusted for. The threshold of SPT 7mm and greater for many foods is associated with a high (>95%) probability that the child is truly food allergic (19). The number of BCG unvaccinated participants in the study was a total of three out of 708 individuals. Of the three, one was positive for sensitisation at an SPT ≥ 7mm. Therefore, the sample size of the unvaccinated group was inadequate to confirm a valid association. In future, a study with a larger unvaccinated (unexposed) group is needed.

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Few studies that have investigated the effect of BCG vaccination on food sensitisation. BCG vaccination has previously been found to decrease the risk for atopy (SPT ≥ 2mm or ≥ 3mm) in other African populations (11, 20), and this protective effect included sensitisation (SPT ≥ 3) to different elements including different foods (11). Further, some studies show a protective relationship between BCG and other atopic disorders such as asthma, eczema and hay fever (11, 12, 20, 21). An atopic individual develops IgE antibodies and elicits allergic symptoms such as atopic dermatitis, allergic rhinitis, asthma and food allergy in response to an antigen (3, 22, 23). There is therefore, a strong link between food allergy and these other forms of atopy. In Cape Town, South Africa, tuberculosis infection in children, measured through a positive tuberculin skin test, was found to be protective for allergic rhinitis (0.43 95% CI: 0.24–0.79) (10). This study, supports the notion that exposure to microbes decreases the risk for allergic diseases, although reverse causation could also have accounted for these findings due to children with atopy being more likely to respond more strongly to the tuberculin skin test. In our study, exposure to the attenuated BCG antigen through vaccination may have decreased the risk of food sensitisation. In support of the protective effect of BCG, a study carried out on mice found that neonatal BCG vaccination resulted in inhibition of allergen induced airway hyper-responsiveness, eosinophilia and mucus over-production (24) and shifted cytokine responses from a predominance of Th2- to Th1-type (24).Therefore, BCG vaccination in mice models was found to be protective for asthma. The studies above provide evidence that BCG vaccination may be protective for food sensitisation, however, the current study cannot confirm this notion.

Limitations of this study include the low number of unvaccinated individuals resulting in poor estimates for the BCG and sensitisation association. Further, no other significant associations were identified due to the low number of unvaccinated participants in the study. Therefore, it is possible that associations may exist but were undetectable. Secondly, the inability to gauge temporality between exposure and outcome due to it being a cross-sectional study. Other studies that have found a significant association between BCG vaccination and atopy were similarly cross sectional (11, 12, 20, 21). Therefore, in future, a cohort study may provide clarity on temporality. Thirdly, when the initial questionnaire was drawn up, details of the time the participant was vaccinated was not sought. Consequently, it was not possible to determine whether the participants received their vaccines according to the South African vaccine schedule or not and if time of vaccination may be playing a role in the attained odds ratios. However, it has previously been shown that time of BCG vaccination does not have an effect on atopy (11), and therefore time of vaccine may not have impacted the association found in the current study. Finally, although a significant association was found between BCG and food sensitisation at a SPT $\geq$ 7mm when the odds ratio was adjusted, there may be residual confounding that was either not measured or not accounted for in the adjusted analyses.

In conclusion, there was very little evidence of an association between BCG vaccination in children and food allergic sensitisation or food allergy. However, in a small subgroup, there was evidence in an association between BCG and SPT $\geq$7 mm.

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# Appendices

# PART D

# Part D: Appendices

## Appendix 1: Vaccine Schedule

| Age of child | EPI schedule (6-10-14 wks) | Private (6-10-14 wks) | Private (6-10-14 wks + HepB at Birth) | Age of child | Option 3 in Private (2-4-6 months) |
|---|---|---|---|---|---|
| **At birth** | OPV(0) | OPV(0) | OPV (0) | **At birth** | OPV(0) |
| | BCG | BCG | BCG | | BCG |
| | | | HBV[1] | | |
| **6 weeks** | OPV(1) | OPV(1) | OPV(1) | **2 months** | OPV(1) |
| | RV (1) | RV (1) | RV (1) | | RV (1) |
| | PCV(1) | PCV(1) | PCV (1) | | PCV (1) |
| | DTaP-IPV/Hib (1)[1] + HBV(1), **or** DTaP-IPV-Hib-HBV (1)[1] | DTaP-IPV-Hib-HBV (1)[2] | DTaP-IPV-Hib-HBV (1)[2] **or** DTaP-IPV-HBV/Hib (1)[2] | | DTaP-IPV-Hib-HBV (1) **or** DTaP-IPV-HBV/Hib (1) |
| **10 weeks** | | RV[3] (2) | RV[3] (2) | **3 or 4 months** | RV[3](2) |
| | | PCV (2) | PCV (2) | | PCV (2) |
| | DTaP-IPV/Hib (2) + HBV(2) **or** DTaP-IPV-Hib-HBV (2) | DTaP-IPV/Hib (2) + HBV (2) **or** DTaP-IPV-Hib-HBV (2) | DTaP-IPV-Hib-HBV (2) **or** DTaP-IPV-HBV/Hib (2) | | DTaP-IPV-Hib-HBV(2) **or** DTaP-IPV-HBV/Hib (2) |
| **14 weeks** | RV (2) | RV[3] (2 or 3) | RV[3] (2 or 3) | **4 or 6 months** | RV[3] (2 or 3) |
| | PCV(2) | PCV (3) | PCV (3) | | PCV (3) |
| | DTaP-IPV/Hib (3) + HBV(3) **or** DTaP-IPV-Hib-HBV (3) | DTaP-IPV/Hib (3) + HBV (3) **or** DTaP-IPV-Hib-HBV (3) | DTaP-IPV-Hib-HBV (3) **or** DTaP-IPV-HBV/Hib (3) | | DTaP-IPV-Hib-HBV(3) **or** DTaP-IPV-HBV/Hib (3) |
| **9 months** | Measles (1) | Measles (1) | Measles (1) | **9 months** | Measles (1) |
| | PCV(3) | MCV (1 | MCV (1) | | MCV (1) |
| **12-15 Months** | | PCV (4)[4] | PCV (4)[4] | **12-15 months** | PCV (4)[4] |
| | | MMR (1) | MMR (1) | | MMR (1) |
| | | Varicella[5] (1) | Varicella[5] (1) | | Varicella[5] (1) |
| | | Hepatitis A (repeat 6 months later) | Hepatitis A (repeat 6 months later) | | Hepatitis A (repeat 6 months later) |
| | | MCV (2) | MCV (2) | | MCV (2) |
| **18 Months** | DTaP-IPV/Hib (4) **or** DTaP-IPV-Hib-HBV (4) | DTaP-IPV/Hib (4) **or** DTaP-IPV-Hib-HBV (4) **or** DTaP-IPV-HBV/Hib (4) | DTaP-IPV/Hib or DTaP-IPV-Hib-HBV (4) or DTaP-IPV-HBV/Hib (4) | **18 months** | DTaP-IPV/Hib or DTaP-IPV-Hib-HBV (3) or DTaP-IPV-HBV/Hib (3) |
| | Measles (2) | | | | |
| **5-6 years** | Td vaccine (**6 years**) | DTaP **or** Tdap -IPV | DTaP **or** Tdap-IPV | **5-6 years** | DTaP **or** Tdap-IPV |
| | | MMR (2) | MMR (2) | | MMR (2) |
| | | Varicella (2) | Varicella (2) | | Varicella (2) |

| | | | | | |
|---|---|---|---|---|---|
| **9 years** | HPV[6] | HPV[7] (from 9 years) | HPV7[8] (from 9 years) | **9 years** | HPV[7]( from 9 years) |
| **12 years** | Td vaccine | Tdap-IPV [8] | Tdap-IPV [8] | **12 years** | Tdap-IPV [8] |

**Abbreviations:**

- **OPV:** Oral polio vaccine,
- **BCG:** Bacille Calmette Guerin vaccine,
- **HBV:** Hepatitis B vaccine,
- **RV:** Rotavirus vaccine
- **Td vaccine:** Tetanus & reduced amount of diphtheria vaccine
- **Tdap-IPV:** (Quadrivalent): Tetanus & reduced amounts of diphtheria and acellular pertussis with inactivated polio vaccine
- **DTaP:** Diphtheria, tetanus and acellular pertussis vaccine
- **DTaP-IPV/Hib:** (Pentavalent): Diphtheria, tetanus, acellular pertussis / inactivated polio & *haemophilus influenzae* type b vaccine
- **DTaP-IPV-Hib-HBV:** (Hexavalent): Diphtheria, tetanus, acellular pertussis / inactivated polio / *haemophilus influenzae* type b and hepatitis B vaccine, fully liquid.
- **DTaP-IPV-HBV/Hib:** (Hexavalent): Diphtheria, tetanus, acellular pertussis / inactivated polio / *haemophilus influenzae* type b and hepatitis B vaccine, requiring constitution
- **HPV:** Human papillomavirus vaccine
- **MCV:** Meningococcal (Groups A, C, W and Y) polysaccharide diphtheria toxoid conjugate vaccine
- **MMR:** Measles, mumps and rubella vaccine ○ **PCV:** Pneumococcal conjugated vaccine

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## Appendix 2: Vaccine Trade Names

| ANTIGEN | TRADE NAME | AGE GROUP |
|---|---|---|
| BCG | BCG® | Usually at birth only but in certain cases up to 1 year |
| CHICKENPOX | VARILRIX® | 9 months and older |
| DTaP | INFANRIX® | 6 weeks to 7th birthday (not usually used for < 2 years of age |
| DTaP-IPV-HBV/Hib<br>DTaP-IPV-Hib-HBV | INFANRIX-HEXA®<br>HEXAXIM® | Children 8 weeks to 2 years<br>6 weeks to 5 years |
| DTaP-IPV/Hib | PENTAXIM® | Children 6 weeks to 2 years. **This product will be phased out in 2015** |
| HEPATITIS A | AVAXIM 80® or HAVRIX JUNIOR® | 1 – 15 years |
| HEPATITIS B (HBV) | HEBERBIOVAC®, EUVAX® or ENGERIX-B® | 0-adulthood (dose according to age) |
| HPV | GARDASIL® (quadrivalent) or<br><br>CERVARIX® (bivalent) | Gardasil ages 9-45 years (girls and women)<br>Gardasil ages 9-26 years (boys and men)<br>Cervarix 9 years and older |
| MCV (A,C,W,Y) | MENACTRA® | 9 months – 23 months; 2 doses 3 months apart.<br>≥ 2years – 55 years – a single dose |
| MEASLES | ROUVAX® | 9 months and older |
| MEASLES, MUMPS, RUBELLA (MMR) | TRIMOVAX® or PRIORIX® | 1 year - adulthood |
| OPV | OPV-MERIEUX® or POLIORAL® | 0-Adulthood (not generally recommended in adulthood due to VAPP – vaccine associated paralytic polio) |
| PNEUMOCOCCAL (PCV) | PREVENAR-13®<br>SYNFLORIX® | Children 6 weeks to 5 years<br>Children 6 weeks to 5 years |
| ROTAVIRUS (RV) | ROTARIX®<br><br>ROTATEQ® | First dose from 6 weeks, second before 24 weeks<br>First dose from 6 weeks of age and by 12 weeks, last dose before 32 weeks |
| Td | DIFTAVAX® | 6 years and older |
| Tdap-IPV | ADACEL QUADRA®<br>BOOSTRIX TETRA® | from 3 Years of age<br>from 4 years of age |

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## Appendix 3: Letter of Approval from HREC

**UNIVERSITY OF CAPE TOWN**
**Faculty of Health Sciences**
**Human Research Ethics Committee**

**Room E52-24 Old Main Building**
**Groote Schuur Hospital**
**Observatory 7925**
**Telephone** [021] 406 6338 • **Facsimile** [021] 406 6411
**Email:** shuretta.thomas@uct.ac.za
**Website:** www.health.uct.ac.za/fhs/research/humanethics/forms

07 March 2016

**HREC REF: 121/2016**

**Prof M Levin**
Paediatrics and Child Health
Room 516, ICH Building
Red Cross Children's Hospital

Dear Prof Levin

**PROJECT TITLE: THE RELATIONSHIP BETWEEN IMMUNISATION AND FOOD ALLERGY AND SENSITISATION IN SOUTH AFRICAN CHILDREN (Masters Candidate - Ms N Ndhlovu)**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until the 30th March 2017.**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

**Please quote the HREC REF in all your correspondence.**

The HREC acknowledge that the student, Nomathamsanqa Ndhlovu will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Yours sincerely

Signed

**PROFESSOR M BLOCKMAN**
**CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE**
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical



Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

## Appendix 4: SAFFA data collection tool

South African Food Sensitisation and Food Allergy (SAFFA) study PID:0000/XX

**Questionnaire completed by:** _____

| | | | |
|---|---|---|---|
| **Q1** | Clinic name | | |
| **Q2** | Enrolment date | DD/MM/YYYY | |
| **Q3** | Study site | 1 = Urban | 2= Rural |
| **Q4** | Date of birth | DD/MM/YYYY | |
| **Q5** | Sex | 1= Male | 2=Female |

| **Crèche visit activity checklist** | | **(please circle)** | |
|---|---|---|---|
| **CK1** | Consent to SAFFA study | Yes/No | If No, please complete non-participant questionnaire |
| **CK2** | | Yes/No | |
| **CK3** | Questionnaire completed | Yes/No | |
| **CK4** | Physical examination completed | Yes/No | |
| **CK5** | Skin Prick Test completed | Yes/No | |
| **CK6** | Need further investigation?<br>• OFC<br>• sIgE | Yes/No | *If OFC:*<br>*Was appointment booked at RXH? Yes/No*<br>*Was parent given OFC information? Yes/No*<br>*Appointment Date:* |
| **CK6a** | Can this participant be a SOSALL control? (BA, Xhosa, No allergies) | 1=Yes | 2=No |

| | | | |
|---|---|---|---|
| **CK 7** | | Yes/No | |
| **CK8** | Attended hospital for OFC | Yes/No | |
| **CK9** | Consent form for OFC and other tests | Yes/No | |
| **CK10** | | Yes/No | |
| **CK11** | | Yes/No | |
| **CK12** | Blood sample indicated (sIgE) | Yes/No | |
| **CK13** | Stool sample indicated (OFC or SOSALL control) | Yes/No | *NB: Also fill in Data Capture sheet no2* |
| **CK14** | Dust sample indicated ( SOSALL Control) | Yes/No | |
| **CK15** | Oral Food Challenge completed and conclusive | Yes/No | If not conclusive,<br>Was repeat appointment booked? Yes/No<br>Appointment Date: |

| | **FINAL RESULT** | | |
|---|---|---|---|
| **CK16** | SPT >1mm | 0 = No | 1 = Yes |
| **CK17** | If SPT >1mm: Is the child tolerant? | 0 = No | 1 = Yes |
| | *If Yes ( tolerant) do serum IgE (CK12)* | | |
| **CK18** | Oral food challenge indicated (SPT>1mm)(not tolerant) | 0 = No | 1 = Yes |
| **CK 15** | OFC completed? | | |

| | | |
|---|---|---|
| **Study Completed as indicated** | 0 = No | 1 = Yes |
| **If NO, is this a non-participant?** | | |
| **Data entry completed** | 0 = No | 1 = Yes |
| **Double data entry completed** | 0 = No | 1 = Yes |

South African Food Sensitisation and Food Allergy (SAFFA) study PID:0000/XX

**Clinic Visit: Physical Examination: Date_____**
**Examined_____**

**Question 6: Anthropometric measures**

| | | | | | |
|---|---|---|---|---|---|
| Q6.1 | Height | cm | Q6.3 | Abdominal girth | cm |
| Q6.2 | Weight | kg | Q6.4 | Skin fold thickness | cm |

**Question 7: Physical examination**

| Q7.1 | Allergic Rhinitis | 0 = No 1 = Yes (tick √) | Q 7.2 | Eczema | 0 = No 1 = Yes (tick √) | Q 7.3 | Asthma | 0 = No 1 = Yes (tick √) |
|---|---|---|---|---|---|---|---|---|
| Sneezing | | | Facial pallor | | | Tachypnoea(>40/min) | | |
| Itchy nose | | | Facial erythema | | | Hyperinflation | | |
| Transverse nasal crease | | | Hypo pigmented patches | | | Prolonged Expiration | | |
| Allergic salute | | | Infra orbital folds (Dennie-Morgan folds) | | | Wheeze | | |
| Rhinorrhoea/discharge | | | Angular cheilitis | | | | | |
| Nasal congestion | | | Anterior neck folds | | | | | |
| Mouth Breathing | | | Flexor involvement | | | | | |
| Oedematous turbinates | | | Extensor patches | | | | | |
| "long face syndrome" | | | Darkening lesions | | | | | |
| Red eyes | | | Lichenification | | | | | |
| Itchy eyes | | | | | | | | |
| Teary eyes/discharge | | | | | | | | |
| Post –nasal drip (Clicking) | | | | | | | | |

**Crèche Visit: Skin Prick Test results: Date_____ Done By_____**
Anti-histamine taken? Yes/No When last?

| Batch Numbers (BOX number: ) | | | | | |
|---|---|---|---|---|---|
| Negative | | Fish(cod) | | Wheat | |
| Egg White | | Peanut | | Soy | |
| Cow's Milk | | Hazelnut | | Positive | |

**Question 8: Skin Prick Test Results (in mm)**

| | Reagent | Weal X mm | Weal Y mm | Weal Mean (mm) | Comments |
|---|---|---|---|---|---|
| 8.1 | Negative | | | | |
| 8.2 | Egg White | | | | |
| 8.3 | Cow's Milk | | | | |
| 8.4 | Soy | | | | |
| 8.5 | Wheat | | | | |
| 8.6 | Fish(cod) | | | | |
| 8.7 | Peanut | | | | |
| 8.8 | Hazelnut | | | | |
| 8.9 | Positive | | | | |
| | **Fresh Agent** | | | | |
| 8.10 | Fresh Peanut | | | | Comments |
| 8.11 | Egg White | | | | |
| 8.12 | Cow's Milk | | | | |
| 8.13 | Der p | | | | |
| 8.14 | German Cockroach | | | | |
| 8.15 | Grass mix | | | | |
| 8.16 | Blomia t | | | | |

### Participant details

| | | | |
|---|---|---|---|
| Q1 | Clinic name | | |
| Q2 | Enrolment date | DD/MM/YYYY | |
| Q3 | Study site | 1 = Urban | 2= Rural |
| Q4 | Date of birth | DD/MM/YYYY | |
| Q5 | Age at enrolment | 00 months | |
| Q6 | Sex | 1= Male | 2=Female |
| Q7 | Weight | 00.0 kg | |
| Q8 | Height/Length | 000 cm | |

### Q9 Immunisations

| | | | Yes | No | | | | Yes | No |
|---|---|---|---|---|---|---|---|---|---|
| Birth | 9.1 | BCG | | | 14 weeks | 9.10 | RV(2) | | |
| | 9.2 | OPV (0) | | | | 9.11 | DTaPIPV/HiB(3) | | |
| 6 weeks | 9.3 | OPV (1) | | | | 9.12 | HepB(3) | | |
| | 9.4 | RV (1) | | | | 9.13 | PCV7 or 13 (2) | | |
| | 9.5 | DTaPIPV/HiB (1) | | | 9 months | 9.14 | Measles vaccine | | |
| | 9.6 | HepB (1) | | | | 9.15 | PCV 7 or 13 (3) | | |
| | 9.7 | PCV7 or 13(1) | | | 18 months | 9.16 | DTaPIPV/HiB (4) | | |
| 10 weeks | 9.8 | DTaPIPV/HiB (2) | | | | 9.17 | Measles Vaccine | | |
| | 9.9 | HepB (2) | | | | | | | |

### Q10 Vaccination Status

| | | | |
|---|---|---|---|
| 1 = Complete | | 2 = Incomplete | |

### Q11 Paracetamol Exposure

| | | | | |
|---|---|---|---|---|
| Q11.1 | Did your child have paracetemol or medicines containing paracetamol in the first year of life? ( Panado, Calpol, Paramed) | | 0 = No | 1 = Yes |
| Q11.2 | If Yes, How old was your child when they first had paracetamol? | | Months | 999 = Don't know |
| Q11.3 | If Yes, How many days did your child have paracetamol in total in the first year of life? | 1 = 1-10 days | 2 = 10-20 days | 3 = More than 20 days |

### Q12 Childhood infections

Has your child had any of the following childhood infections?

| | | 0 = No | 1 = Yes | 999 = don' t know | | | 0 = No | 1 = Yes | 999 = don't know |
|---|---|---|---|---|---|---|---|---|---|
| 12.1 | Measles | | | | 12.5 | Glandular Fever | | | |
| 12.2 | Mumps | | | | 12.6 | Tuberculosis | | | |
| 12.3 | Rubella | | | | 12.7 | Hepatitis | | | |
| 12.4 | Chickenpox | | | | 12.8 | Other (please specify) | | | |

South African Food Sensitisation and Food Allergy (SAFFA) study PID:0000/XX

## Q13 Antibiotic and anti-helminthic exposure

| | | | | | |
|---|---|---|---|---|---|
| 13.1 | Did your child have any antibiotics in the first year of life? | 0 = no | 1 = Yes | 999 = Don't know | |
| 13.2 | If Yes, how old were they with the first course?? | | Months | 999=Don't know | |
| 13.3 | How many courses did your child have in the first year of life? | 1 = None | 2 = 1 to 2 Courses | 3 = 3 to 5 courses | 4 = > 5 courses |
| 13.4 | Did your child have any antibiotics in the last 2 months? | 0 = No | 1 = Yes | 999 = Don't know | |

| | | | | |
|---|---|---|---|---|
| 13.5 | Has your child ever had medicines for worms? | 0= No | 1 = Yes | 999=Don't Know |
| 13.6 | If Yes, at what age were they first dewormed? | | months | 999=Don't know |
| 13.7 | If Yes, at what age did they last take anti worm medicines? | | months | 999=Don't know |
| 13.8 | Has your child had regular (yearly) medicine for worms? | 0 = No | 1 = Yes | 999 = Don't know |

## Q14 Probiotic Exposure and *Amasi* Exposure (CHILD)

| | | | | |
|---|---|---|---|---|
| 14.1 | Did this **child** have probiotics in food or supplements in the first year of life? | 0 = No | 1 = Yes | 999 = Don't know |
| 14.2 | If Yes, how old was this child when they first had probiotics? | | | Months |
| 14.3 | If Yes, How many days in total did this child have probiotics in the first year of life? | 1 = 1-10 days | 2 = 10-20 days | 3 = >20 days |
| 14.4 | If Yes, which probiotic product were they given? | | | |

| | | | | |
|---|---|---|---|---|
| 14.3 | Has your **child** ever have **amasi** ? | 0 = No | 1 = Yes | 999 = Don't know |
| 14.4 | If Yes, how old was this child when they first had **amasi**? | | | Months |
| 14.5 | If Yes, how often does your child take amasi? | 1 = Less than once a month | 2 = 1-4 times per month | 3 = more than 4 times per month |

## Probiotic and *Amasi* exposure (MOTHER)

| | | | | |
|---|---|---|---|---|
| 14.6 | Did this child's mother have probiotics during pregnancy? | 0 = No | 1 = Yes | 999 =Don't know |
| 14.7 | If Yes, How many days in total did she use probiotics? | 1 = 1-10 days | 2 = 10-20 days | 3 = >20 days |
| 14.8 | Which yes, which products did she use? | | | |
| 14.9 | Did this child's mother regularly have amasi during pregnancy? (**more than once a month**) | 0 = No | 1 =Yes | 999 = Don't know |

## Q15 Delivery mode

| | | | | |
|---|---|---|---|---|
| Q15 | How was this child born? | 1 = Normal vaginal delivery | 2 = Caesarean section | 999 = Don't Know |

South African Food Sensitisation and Food Allergy (SAFFA) study PID:0000/XX

## Q16 Sunlight Exposure

How much time does your child spend outdoors on an average day in winter and in summer?

*Prompts:*
*How many days per week does your child attend crèche?*
*Does your child play outside before leaving for crèche?*
*Do you walk to crèche? How many minutes. What time do you drop off and pick up your child*
*Crèche scheduled play time*
*Does your child play outside after crèche?*
*On Saturdays and Sundays: walk to shops/parks, walk to church, plays outside, and comes in for lunch/nap time?*

*[ (weekdays before and after crèche + crèches playtime)x5 +Saturday +Sunday]÷7*

| Q16.1 Winter | Q16.2 Summer |
|---|---|
| hours | hours |
| 888 = Don't Know | 888 = Don't Know |

## Q17 Peanut exposure in pregnancy

| | | | | |
|---|---|---|---|---|
| 17.1 | Did this child's mother eat peanuts regularly **(at least once a week)** during pregnancy? | 0 = No | 1 = Yes | 999 = Don't know |
| 17.2 | Did this child's mother completely avoid peanuts during pregnancy? | 0 = No | 1 = Yes | 999 = Don't know |

## Q18 Breastfeeding information

| | | | | |
|---|---|---|---|---|
| Q18.1 | Was this child ever breastfed? | 0 = No | 1 = Yes | 999 = Don't know |
| Q18.2 | If Yes, up to what age was this child exclusively breastfed (i.e. no milk or other fluids via bottle nor given any solids) | | Months | 999 = Don't know |
| Q18.3 | At what age did you completely stop breastfeeding this child? | | Months | 999 = Don't know<br>Still breastfeeding |
| Q18.4 | At what age did you first introduce any other milk (other than breast milk) | | Months | 999 = Don't know |

## Q19 Weaning foods

| | | | | |
|---|---|---|---|---|
| Q19.1 | When did you first introduce food into your child's diet? | Months | | 999=Don't know |
| Q19.2 | Which three types of foods did you introduce first? *(see coding chart for food categories)* | | | |

## Q20 Exposure to Unpasteurised milk

| | | | | |
|---|---|---|---|---|
| Q20 | Has this child ever had non-pasteurised (Fresh farm) milk? | 0 = No | 1 = Yes | 999 = Don't know |

## Q21 Food exposure

| Foods Eaten | | Ever Eaten | | | Age first eaten | | | | Still eating regularly (once a month or more) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21.1 | **Peanut** e.g. peanut butter, peanuts in cookies or chocolates | 21.1.1 | 0 = No | 1 = Yes | 21.1.2 | | months | 999 = don't know | 21.1.3 | 0 = No | 1 = Yes **If yes please continue with 21.1.4** |

**If yes to 21.1.4**

**Please try an remember how many times your child ate the following peanut containing foods in the last 7 days**

| Type of peanuts consumed | Number of times per day | Number of times per week | Usual amount | | | Total amount in grams |
|---|---|---|---|---|---|---|
| Peanut butter on bread | | | | Thin slices | 21.1.4 | |
| | | | | Thick slices | 21.1.5 | |
| Peanut butter in porridge | | | | Teaspoons | 21.1.6 | |
| Raw/boiled peanuts | | | | Handfuls | 21.1.7 | |
| Roasted peanuts | | | | Handfuls | 21.1.8 | |
| Peanuts in chocolate or biscuits | | | | | 21.1.9 | |
| Total amount of peanut consumed in the last 7 days | | | | | 21.1.10 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21.2 | **Other nuts** e.g. Cashew, hazelnut, brazil nut, almonds, walnuts, pistachio, macadamia in chocolate and cookies or cakes and breads | 21.2.1 | 0 = No | 1 = Yes | 21.2.2 | | months | 999 = don't know | 21.2.3 | 0 = No | 1 = Yes |
| 21.3 | **Cow's milk products** e.g.yoghurt, amasi, ,custard, cheese, yoghurt, cream, fresh milk | 21.3.1 | 0 = No | 1 = Yes | 21.3.2 | | months | 999 = don't know | 21.3.3 | 0 = No | 1 = Yes |
| 21.4 | **Cow's milk formula** e.g. Nan, Lactogen, Novolac, S26, Infacare, Pelargon | 21.4.1 | 0 = No | 1 = Yes | 21.4.2 | | Months | 999 = don't know | 21.4.3 | 0 = No | 1 = Yes |
| 21.5 | **Soya products** e.g. soya mince, , tofu, baby foods, soya in bread | 21.5.1 | 0 = No | 1 = Yes | 21.5.2 | | Months | 999 = don't know | 21.5.3 | 0 = No | 1 = Yes |
| 21.6 | **Soya milk products** e.g. Infasoy, Isomil, Infacare | 21.6.1 | 0 = No | 1 = Yes | 21.6.2 | | Months | 999 = don't know | 21.6.3 | 0 = No | 1 = Yes |
| 21.7 | **Hen's egg** e.g. boiled, scrambles eggs, omelettes, French toast, quiches | 21.7.1 | 0 = No | 1 = Yes | 21.7.2 | | Months | 999 = don't know | 21.7.3 | 0 = No | 1 = Yes |
| 21.8 | **Wheat** e.g. cereal (Wheetabix, All Bran, Tasty Wheat), noodles, bread or pasta, cakes, rusks, couscous, semolina | 21.8.1 | 0 = No | 1 = Yes | 21.8.2 | | Months | 999 = don't know | 21.8.3 | 0 = No | 1 = Yes |
| 21.9 | **Fish (excluding shellfish)** Hake, snoek, sardines,pilchards, tuna, kingklip, salmon etc and fish products: Fish paste(Reidro), Worcester sauce, fish sauce | 21.9.1 | 0 = No | 1 = Yes | 21.9.2 | | months | 999 = don't know | 21.9.3 | 0 = No | 1 = Yes |

## Q22 Food Reactions

| 22.1 PEANUTS | | | | | |
|---|---|---|---|---|---|
| 22.1.1 | Has your child ever had any of these reactions below to peanuts or food containing peanuts | | | 0 = No | 1 = Yes |
| 22.1.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1= none | 6= tight throat | 11=shock/low blood pressure | | |
| | 2= Itchy Rash | 7= wheeze | 12= collapse/loss of consciousness | | |
| | 3=swelling(face/lips/eyes) | 8= vomiting | 13 = worsening of eczema | | |
| | 4= flushing | 9= diarrhoea | | | |
| | 6= itchy mouth/throat | 10= blue lips | | | |
| 22.1.3 | If Yes, how old was this child when he/she had the first reaction? | | | Months | |
| 22.1.4 | If yes, how long after the eating the food did the reaction occur? | | 1 = < 1 hour | 2 = 1-24 hours | 3 = >24 hours |
| 22.1.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | 1 = Yes | |

| 22.2 OTHER NUTS | | | | | |
|---|---|---|---|---|---|
| 22.2.1 | Has your child ever had ny of these reactions below to other nuts or food containing nuts other than peanuts | | | 0 = No | 1 = Yes |
| 22.2.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1 = none | 6 = tight throat | 11 =shock/low blood pressure | | |
| | 2 = Itchy Rash | 7 = wheeze | 12 = collapse/loss of consciousness | | |
| | 3 =swelling(face/lips/eyes) | 8 = vomiting | 13 = worsening of eczema | | |
| | 4 = flushing | 9 = diarrhoea | | | |
| | 6 = itchy mouth/throat | 10 = blue lips | | | |
| 22.2.3 | If Yes, how old was this child when he/she had the first reaction? | | | Months | |
| 22.2.4 | If yes, how long after the eating the food did the reaction occur? | | 1 = < 1 hour | 2 = 1-24 hours | 3 = >24 hours |
| 22.2.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | 1 = Yes | |

| 22.3 COW'S MILK | | | | | |
|---|---|---|---|---|---|
| 22.3.1 | Has your child ever had any of these reactions below to cow's milk or food containing cow's milk | | | 0 = No | 1 = Yes |
| 22.3.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1 = none | 6 = tight throat | 11 = shock/low blood pressure | | |
| | 2 = Itchy Rash | 7 = wheeze | 12 = collapse/loss of consciousness | | |
| | 3 =swelling(face/lips/eyes) | 8 = vomiting | 13 = worsening of eczema | | |
| | 4 = flushing | 9 = diarrhoea | | | |
| | 6 = itchy mouth/throat | 10 = blue lips | | | |
| 22.3.3 | If Yes, how old was this child when he/she had the first reaction? | | | Months | |
| 22.3.4 | If yes, how long after the eating the food did the reaction occur? | | 1 = < 1 hour | 2 = 1-24 hours | 3 = >24 hours |
| 22.3.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | 1 = Yes | |

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| 22.4 SOYA | | | | | |
|---|---|---|---|---|---|
| 22.4.1 | Has your child ever had any of these reactions below to soya or food containing soya | | | 0 = No | 1 = Yes |
| 22.4.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1 = none | 6= tight throat | 11=shock/low blood pressure | | |
| | 2 = Itchy Rash | 7= wheeze | 12= collapse/loss of consciousness | | |
| | 3 =swelling(face/lips/eyes) | 8= vomiting | 13 = worsening of eczema | | |
| | 4 = flushing | 9= diarrhoea | | | |
| | 5 = Itchy mouth/throat | 10= blue lips | | | |
| 22.4.3 | If Yes, how old was this child when he/she had the first reaction? | | | | Months |
| 22.4.4 | If yes, how long after the eating the food did the reaction occur? | | 1 = < 1 hour | 2 = 1-24 hours | 3 = >24 hours |
| 22.4.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | | 1 = Yes |

| 22.5 HEN'S EGG | | | | | |
|---|---|---|---|---|---|
| 22.5.1 | Has your child ever had any of these reactions below to hen's egg or food containing egg | | | 0 = No | 1 = Yes |
| 22.5.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1 = none | 6 = tight throat | 11 =shock/low blood pressure | | |
| | 2 = Itchy Rash | 7 = wheeze | 12 = collapse/loss of consciousness | | |
| | 3 =swelling(face/lips/eyes) | 8 = vomiting | 13 = worsening of eczema | | |
| | 4 = flushing | 9 = diarrhoea | | | |
| | 5 = Itchy mouth/throat | 10= blue lips | | | |
| 22.5.3 | If Yes, how old was this child when he/she had the first reaction? | | | | months |
| 22.5.4 | If yes, how long after the eating the food did the reaction occur? | | 1= < 1 hour | 2= 1-24 hours | 3= >24 hours |
| 22.5.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | | 1 = Yes |

| 22.6 WHEAT | | | | | |
|---|---|---|---|---|---|
| 22.6.1 | Has your child ever had any of these reactions below to wheat or food containing wheat | | | 0 = No | 1 = Yes |
| 22.6.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1= none | 6= tight throat | 11=shock/low blood pressure | | |
| | 2= Itchy Rash | 7= wheeze | 12= collapse/loss of consciousness | | |
| | 3=swelling(face/lips/eyes) | 8= vomiting | 13 = worsening of eczema | | |
| | 4= flushing | 9= diarrhoea | | | |
| | 5= Itchy mouth/throat | 10= blue lips | | | |
| 22.6.3 | If Yes, how old was this child when he/she had the first reaction? | | | | Months |
| 22.6.4 | If yes, how long after the eating the food did the reaction occur? | | 1= < 1 hour | 2= 1-24 hours | 3= >24 hours |
| 22.6.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | | 1 = Yes |

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| 22.7 FISH (excluding shellfish) | | | | | |
|---|---|---|---|---|---|
| 22.7.1 | Has your child ever had any of these reactions below to fish or food containing fish | | | 0 = No | 1 = Yes |
| 22.7.2 | If yes, which of the following reactions has this child had? (you can circle more than one) | | | | |
| | 1 = none | 6 = tight throat | 11 =shock/low blood pressure | | |
| | 2 = Itchy Rash | 7 = wheeze | 12 = collapse/loss of consciousness | | |
| | 3 =swelling(face/lips/eyes) | 8 = vomiting | 13 = worsening of eczema | | |
| | 4 = flushing | 9 = diarrhoea | | | |
| | 5 = itchy mouth/throat | 10 = blue lips | | | |
| 22.7.3 | If Yes, how old was this child when he/she had the first reaction? | | | | Months |
| 22.7.4 | If yes, how long after the eating the food did the reaction occur? | | 1= < 1 hour | 2= 1-24 hours | 3= >24 hours |
| 22.7.5 | If yes, was this reaction confirmed by a doctor? | | 0 = No | | 1 = Yes |

## Q23 Asthma

| | | | | |
|---|---|---|---|---|
| Q23.1 | Has your child ever symptoms of asthma without having a cold or chest infection? (e.g. wheeze, persistent cough at night or when exercising, shortness of breath) | 0 = No | | 1 = Yes |
| Q23.2 | If Yes, how old was he/she? | | | Months |
| Q23.3 | If yes, who diagnosed the asthma? | 1 = Self | 2 = Nurse | 3 = Doctor |

## Q24 Hay fever

| | | | | |
|---|---|---|---|---|
| Q24.1 | Has your child ever had symptoms of hay fever (e.g. itchy runny eyes, itchy runny nose, blocked nose, frequent sneezing) without having a "cold" or upper respiratory tract infection? | 0 = No | | 1 = Yes |
| Q24.2 | If Yes, how old was he/she? | | | Months |
| Q24.2 | If yes, who diagnosed the hay fever? | 1 = Self | 2 = Nurse | 3 = Doctor |

## Q25 Eczema

| | | | | |
|---|---|---|---|---|
| Q25.1 | Has your child ever had symptoms of eczema (e.g. an itchy rash especially in the folds of the elbows, behinds the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes?) | 0 = No | | 1 = Yes |
| Q25.2 | If yes, how old was your child then? | | | Months |
| Q25.3 | If yes, who diagnosed the eczema? | 1 = Self | 2 = Nurse | 3 = Doctor |

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## Q26 Medication use

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Q26.1 | Is your child on any of the following medication? (see NAEP Chart) | | | | | | | |
| 26.1.1 | INHALERS | Relievers (blue) e.g. Asthavent | 0 = No | 1 = Yes | 25.1.5 | Nasal corticosteroid spray e.g. Beclate | 0 = No | 1 = Yes |
| 26.1.2 | | Controllers (brown/cream) e.g. Budeflam | 0 = No | 1 = Yes | 25.1.6 | Antihistamines (If yes, please complete Q28.4 below) | 0 = No | 1 = Yes |
| 26.1.3 | | Other (please specify below) | 0 = No | 1 = Yes | 25.1.7 | Steroid creams | 0 = No | 1 = Yes |
| 26.1.4 | | Home nebuliser | 0 = No | 1 = Yes | 25.1.8 | Adrenalin auto injector or pen e.g. Epipen | 0 = No | 1 = Yes |
| Q26.2 | If your child is on any other oral medication (pills or syrups) for asthma, please specify each one. (e.g. oral steroids or leukotriene receptor antagonists) | | | | Free text | | | |
| Q26.3 | If your child is on antihistamines, please specify which type/brand? | | | | Free text | | | |
| Q26.4 | If your child is on antihistamines, how many days since they were last taken? | | | | 1 = <2 days | 2 = 2-5 days | 3 = >5 days | |
| Q27.5 | If your child is on any other medication for other illnesses, please specify each one. | | | | Free text | | | |

## Q27 Family history of allergic disease

| | Does anyone in your family have allergic diseases? Please circle. (you can choose more than one option) | | | | | |
|---|---|---|---|---|---|---|
| | Family member | None | Asthma | Hay fever | Eczema | Food allergy |
| Q27.1 | Mother | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |
| Q27.2 | Father | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |
| Q27.3 | Full Sibling 1 | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |
| Q26.4 | Full Sibling 2 | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |
| Q27.5 | Full Sibling 3 | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |
| Q27.6 | Full Sibling 4 | 1 = none | 2 = asthma | 3 = hay fever | 4 = eczema | 5 = food allergy |

## Q28 Child's Medical history

| | | | |
|---|---|---|---|
| Q28.1 | Does your child have any other significant medical problems? (e.g. heart or lung problems, kidney or liver disease, epilepsy, diabetes) | 0 = No | 1 = Yes |
| Q28.2 | If yes, please specify | | |

## Q29 Home Language and Migration

| | | | | |
|---|---|---|---|---|
| Q29.1 | What language do you mainly speak at home? (Please choose only one) | | | |
| | 1 = IsiXhosa | 2 = English | 3 = Afrikaans | |
| | 4 = IsiZulu | 5 = Sesotho | 6 = Setswana | |
| | 7 = SiSwati | 8 = IsiNdebele | 9=Xitsonga | |
| | 10 = Sepedi | 11=Tshivenda | 12 = Other | |
| Q29.1.2 | If Xhosa speaking, was this child born in Cape Town? | | 0 = No | 1 = Yes |

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| | | | | | |
|---|---|---|---|---|---|
| 29.2.1 | If No, where was this child born? | Province | | | |
| | | 1 = Western Cape<br>2 = Eastern Cape<br>3 = Northern Cape<br>4 = Free state<br>5 = Gauteng | | 6 = Kwazulu Natal<br>7 = Mpumalanga<br>8 = Limpopo<br>9 = North West Province<br>10 = Other | |
| | | Town (specify) | | Free text | |
| 29.2.2 | When did this child move to Cape Town? | | Year | | Month |

| | | | | | |
|---|---|---|---|---|---|
| Q29.3 | Was this child's **MOTHER** born in Cape Town? | | | 0 = No | 1 = Yes |
| 29.3.1 | If No, where was she born? | Province (please circle) | | | |
| | | 1 = Western Cape<br>2 = Eastern Cape<br>3 = Northern Cape<br>4 = Free state<br>5 = Gauteng | | 6 = Kwazulu Natal<br>7 = Mpumalanga<br>8 = Limpopo<br>9 = North West Province<br>10 = Other | |
| | | Town (specify) | | Free text | |
| 29.3.2 | If no when did SHE move to Cape Town? | | Year | | Month |
| 29.3.3 | Was this child's **FATHER** born in Cape Town? | | | 0 = No | 1 = Yes |
| | If No, where was he born? | Province | | | |
| | | 1 = Western Cape<br>2 = Eastern Cape<br>3 = Northern Cape<br>4 = Free state<br>5 = Gauteng | | 6 = Kwazulu Natal<br>7 = Mpumalanga<br>8 = Limpopo<br>9 = North West Province<br>10 = Other | |
| | | Town(specify) | | | |
| 29.3.4 | If no when did HE move to Cape Town? | | Year | | Month |

## Q30 Ethnicity

| | | | |
|---|---|---|---|
| Q30 | What is your child's ethnic origin? (circle as appropriate) | 1 = White/Caucasian | 2 = Coloured / Mixed race |
| | | 3 = Black African | 4 = Asian/Indian |
| | | 5 = Other (Specify) | |

## Q30 Household information

| | | |
|---|---|---|
| Q31.1 | How many people live together in your house? | |
| Q31.2 | How many children (12 years or less) that are OLDER than this child live in the same household? | |
| Q31.3 | How many children that are YOUNGER than this child live in the same household? | |

## Q32 Parental Education level

| | |
|---|---|
| **1** = None | **11** = Grade 9 / Std 7 |
| **2** = Grade R / preschool | **12** = Grade 10 / Std 8 |
| **3** = Grade 1 / SubA | **13** = Grade 11 / Std 9 |
| **4** = Grade 2 / Sub B | **14** = Grade 12 / Matric |
| **5** = Grade 3 / Std 1 | **15** = Grade 9,10,11 (Std 7,8,9) & diploma |
| **6** = Grade 4 / Std 2 | **16** = Grade 12 (Std 10) & Certificate or Diploma |
| **7** = Grade 5 / Std 3 | **17** = Grade 12 (Std 10) & Degree |
| **8** = Grade 6 / Std 4 | **18** = Grade 12 (Std 10) & Degree plus Diploma or further degree |

### Q33 Household income

| | | | | |
|---|---|---|---|---|
| Q33.1.1 | Is this child's mother/female guardian:<br>(please choose 1 option) | 1=student<br>2=unemployed | 3=employed<br>999 = Don't know | |
| Q33.1.2 | If employed, what job does this she do? | | | 999=Don't Know |
| Q33.2.1 | Is this child's father/male guardian:<br>(please choose 1 option) | 1 = Student<br>2 = Unemployed | 3 = Employed<br>999=Don't know | |
| Q33.2.2 | If employed, what job does he do? | | | 999= Don't Know |
| Q33.3 | How much money or income does your household receive every month after tax? (incl. money from work, pensions, informal business etc.) | R | | 999 = Don't know |

### Q34 Contact with pets and animals

| | | | | |
|---|---|---|---|---|
| Q34.1 | Do you own a cat or have a cat in your home? | | 0 = No | 1 = Yes |
| Q34.2 | Do you own a dog or have a dog in your home? | | 0 = No | 1 = Yes |
| Q34.3 | Does your child have regular (at least once a week) contact with farm animals (e.g. cattle, pigs, goats, sheep or poultry)? | 0 = No | 1 = Yes | 999 = Don't know |
| Q34.4 | Has this child's mother had regular (at least once a week) contact with farm animals (e.g. cattle, pigs, goats, sheep or poultry) while being pregnant with this child? | 0 = No | 1 = Yes | 999 = Don't know |

### Q35 Fuel exposure

| | | |
|---|---|---|
| Q35.1 | At your house, what fuel is used for cooking? | |
| | 1 = Electricity/Gas | 4 = Open fires outside the house |
| | 2 = Paraffin Stove | 5 = Other (specify) |
| | 3 = Open fires in the house | |
| Q35.2 | At your house, what fuel is used for heating? | |
| | 1 = Electricity | 4 = Wood/coal |
| | 2 =Gas | 5 = Other (specify) |
| | 3 =Kerosene/Paraffin | |

### Q36 Cigarette smoke exposure

| | | | | |
|---|---|---|---|---|
| Q36.1 | Does this child's mother (or female guardian) currently smoke cigarettes? | 0 = No | 1 = Yes | 999 = Don't know |
| 36.1.1 | If **YES**, about how many cigarettes does the child's mother (or female guardian) smoke each day? | number of cigarettes: | | |
| Q36.2 | Does this child's father (or male guardian) currently smoke cigarettes? | 0 = No | 1 = Yes | 999= Don't know |
| 36.2.1 | If **YES**, about how many cigarettes does the child's father (or male guardian) smoke each day? | number of cigarettes: | | |
| Q36.3 | How many people living in the house currently smoke cigarettes, including parents? | No of people: | | |
| Q36.4 | Did this child's mother smoke cigarettes while being pregnant with this child? | 0 = No | 1 = Yes | 999 = Don't know |

| | | | | | |
|---|---|---|---|---|---|
| **Q37** | **How often does your child eat fast foods?** | | | | |
| | 1=<br><1 a month | 2=<br>1-3 a month | 3=<br>1-2 x a week | 4=<br>3-4 x a week | 5=<br>>5 x a week |
| **Q38** | **How often does your child drink soft drinks and fruit juices?**<br>*(except pure orange juice)* | | | | |
| | 1=<br><1 a month | 2=<br>1-3 a month | 3=<br>1-2 x a week | 4=<br>3-4 x a week | 5=<br>>5 x a week |
| **Q39** | **How many pieces of fruit and vegetables has your child eaten in the last 48 hours?** | | | | |
| | 1=<br><1 | 2=<br>2-3 | 3=<br>4-5 | 4=<br>6+ | |
| **Q40** | **How often does your child have fried or microwaved sources of meat?** | | | | |
| | 1=<br><1 a month | 2=<br>1-3 a month | 3=<br>1-2 x a week | 4=<br>3-4 x a week | 5=<br>>5 x a week |

## Appendix 5: Instructions from the Annals of Allergy, Asthma & Immunology for manuscript

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**General Information**

The Annals of Allergy, Asthma & Immunology is the official journal of the American College of Allergy, Asthma and Immunology. The Annals acceptance rate is approximately 32%. Median turnaround time from submission to first decision is 22 days, from submission to acceptance is 78 days, and acceptance to publication is 86 days.

Authors are responsible for all statements, opinions, conclusions, and methods of presenting their data in articles submitted to the Annals of Allergy, Asthma & Immunology for possible publication. The views of authors as presented in their articles do not necessarily represent the opinions of the Annals of Allergy, Asthma & Immunology editorial staff or the American College of Allergy, Asthma and Immunology.

The purpose of these instructions is to provide authors with clear and concise guidelines for preparing a manuscript in acceptable Annals style. In general, exceptions to the published guidelines are not made. Authors who believe they have compelling reasons to alter and/or exceed the published guidelines may appeal to the editorial office for a variance PRIOR TO submission. Appeals may be sent to annallergy@umc.edu. Otherwise, manuscripts that do not meet these guidelines will be returned to the corresponding author for revision prior to any further consideration for peer review.

**Editorial Policies for Authors**

*Authorship*

It is assumed that a submitted manuscript is the work of the listed authors and represents the effort to generate the manuscript. While outside editorial assistance may be utilized, "ghost written" articles are not accepted for review by the Annals. By submitting a manuscript the authors certify that they have (collectively) personally written at least 90 percent of the manuscript.

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In order to be included in the list of authors, an individual must meet all the following requirements approved by the International Committee of Medical Journal Editors (ICMJE): (1) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafted the article or reviewed it critically for important intellectual content; (3) given final approval of the version to be published; and (4) agrees to be accountable for all aspects of the work related to its accuracy or integrity.

*Manuscript Preparation*

NOTE: Manuscripts that do not adhere to the following requirements will be returned to the corresponding author before peer review is initiated.

Basic Formatting (Page Setup/Fonts)

Each manuscript submission item, as described later in this document, should be formatted as follows (unless otherwise specified in the Article Type/Organization section of this document):

1. Be in a standard font such as Times New Roman, Arial, or Courier, size 12,

Part D Appendices

2. Be attached as a separate submission item,

3. Be double-spaced and have a one inch margin on all side, and

4. Display page numbers in the upper right corner of each page and continuous Line numbers (i.e., 1, 2, 3 etc.) in the left-hand margin of the Manuscript and Reference Submission items. Do NOT restart numbering from each page. Line numbering can be added from the File/Page Setup menu of word processing programs and should be continuous throughout the manuscript text file, and

5. File names should NOT contain brackets [ ].

**Abstracts**

Original research, systematic reviews, and meta-analyses require structured abstracts. Structured abstract should not exceed 255 words. Abstracts should NOT be included on the title page, but recorded during the online submission process. Please refer to the Article Type/Organization section of this document for the structured abstract formats.

**Manuscript Text File**

All submissions require a manuscript text file, unless otherwise specified in the Article Type/Organization section of this document.

The text file should display page numbers in the upper right corner of each page and continuous line numbers (i.e., 1, 2, 3 etc.) in the left-hand margin of the submission item. Do NOT restart numbering from each page. Line numbering can be added from the File/Page Setup menu of word processing.

In order to ensure a double-blind review, it is imperative that author identifying information not be included in the manuscript text. Abstracts, acknowledgments, E-supplement material, figures, tables and references should NOT be included in the manuscript text file.

Text should be divided into the following section headings in this order, unless otherwise specified in the Article Type/Organization section of this document.

**Introduction**: Provide an overview of the scope and relevance of the study. The introduction should be brief and state the problem being investigated, its contextual background, and the reasons for conducting the research.

**Methods**: Describe the design (randomized, double-blind, placebo control), subjects, setting (general community, private practice, and hospital), interventions, and main outcome measures. For all research studies involving animal or human subjects or research material derived from humans, appropriate institutional review board (IRB) review and approval is required and the manner in which informed consent was obtained from the study participants (i.e., oral or written) should be stated in the Methods section of the manuscript. Studies exempted from IRB approval by their respective boards should be stated in the Methods section. If no formal ethics committee is available, authors should indicate in the Methods section that procedures followed were in accordance with the Helsinki Declaration as revised in 2013 ( http://www.wma.net/en/30publications/10policies/b3/). When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed. Such review and approval or waiver should also be stated in the Methods sections of the manuscript.

**Results**: Describe the experimental data and results as well as the particular statistical significance of the data.

**Discussion**: Provide and quantify the main outcomes of the study. Identify limitations of the presented data including plausible explanations for discrepancies between the data and the literature, any differences not expected from the initial hypothesis presented in the introduction and a measured

description of the conclusions of the study with implications for future research, biological understanding and/or clinical applications.

For guidance regarding grammar, punctuation, and scientific writing see the AMA Manual of Style, 10th ed. New York: Oxford Press; 2007.

**Acknowledgements**

All persons who have made substantial contributions to the work reported in the manuscript (e.g., data collection, analysis, or writing or editing assistance) but who do not fulfill the authorship criteria should be named with their specific contributions in the Acknowledgments section.

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5. Journal names should be abbreviated according to Index Medicus.

6. All authors up to 6 should be listed; if there are more than 6 authors, list the first 3 followed by "et al."

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Tables should be prepared using Microsoft Word. Each table accompanying a submission should be uploaded as a separate submission item. Tables should:

1. Be numbered in the order in which they are first cited in the text.

2. Be recorded on the title page and during the electronic submission process.

3. Have a concise heading (no more than 30 words).

4. Be comprehensible without reference to the text of the article. Use horizontal lines only at the top and bottom of the table and between column headings and the body of the table. Use no vertical lines.

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6. Abbreviations should be defined in alphabetical order at the bottom of the table, e.g., Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; OR, odds ratio.

Part D Appendices

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Original articles should have a structured abstract of 255 words or less with the following headings: Background, Objective, Methods, Results, and Conclusion. A maximum of 12 keywords, 60 references, and a combined total of 8 tables and/or figures are allowed. Text should not exceed 4,000 words and should be organized into the following headings: Introduction, Methods, Results, and Discussion. Each submission must be comprised of the following submission items:

1. Cover letter

2. Title page

3. Manuscript

4. Acknowledgments, if applicable

5. References

6. Figures, if applicable

7. Figure legend, if applicable

8. Tables, if applicable

9. eSupplements, if applicable

## Part D Appendices

**Appendix 6: Table: Associations between immunisation status and SPT≥ 1mm, ≥ 3mm and ≥ 7mm in urban participants**

| Outcome | Vaccine | OR | 95% CI | *adjusted OR | 95%CI |
|---|---|---|---|---|---|
| **SPT ≥ 1** | BCG | 0.3 | 0.03 - 3.4 | 0.2 | 0.02 - 2.7 |
| | OPV(0) | 0.3 | 0.06 - 1.7 | 0.3 | 0.1 - 1.5 |
| | OPV(1) | 1 | | | |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 1 | | | |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1.6 | 0.2 - 12.3 | 1.8 | 0.2 - 14.9 |
| | PCV(3) | 1.01 | 0.2 – 4.5 | 1.1 | 0.2 - 5.0 |
| | DTaP-IPV/Hib (4) | 1 | 0.4 - 2.5 | 0.9 | 0.4 - 2.4 |
| | MV2 | 1 | 0.4 - 2.5 | 0.9 | 0.4 - 2.4 |
| **SPT ≥ 3** | BCG | 0.2 | 0.02 – 2.4 | 0.2 | 0.02 - 2.7 |
| | OPV(0) | 0.2 | 0.04 – 1.2 | 0.3 | 0.1 - 1.5 |
| | OPV(1) | 1 | | | |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 1 | | | |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1.1 | 0.1 - 8.6 | 1.8 | 0.2 - 14.9 |
| | PCV(3) | 0.7 | 0.2 - 3.1 | 1.1 | 0.2 - 5.0 |
| | DTaP-IPV/Hib (4) | 0.8 | 0.3 – 2.2 | 0.8 | 0.3 - 2.3 |
| | MV2 | 0.8 | 0.3 – 2.2 | 0.8 | 0.3 - 2.3 |
| **SPT ≥ 7** | BCG | **0.09** | **0.08 - 0.97** | **0.05** | **0.004 - 0.6** |
| | OPV(0) | 0.2 | 0.02 - 1.9 | 0.2 | 0.02 - 1.6 |
| | OPV(1) | 1 | | | |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |

| | | | | | |
|---|---|---|---|---|---|
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 1 | | | |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 0.4 | 0.1 - 3.5 | 0.5 | 0.1 - 4.6 |
| | PCV(3) | 0.6 | 0.1 - 4.8 | 0.7 | 0.1 - 5.7 |
| | DTaP-IPV/Hib (4) | 0.8 | 0.2 – 3.5 | 0.6 | 0.1 - 3.1 |
| | MV2 | 0.8 | 0.2 – 3.5 | 0.6 | 0.1 - 3.1 |

**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema*

## Appendix 7: Table: Association between immunisation status and food allergy in the urban participants

| **Outcome** | **Vaccine** | **OR** | **95% CI** | ***adjusted OR** | **95%CI** |
|---|---|---|---|---|---|
| **Food allergy** | BCG | 1 | | | |
| | OPV(0) | 0.2 | 0.02 - 1.8 | 0.1 | 0.02 - 1.3 |
| | OPV(1) | 1 | | | |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 1 | | | |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 0.6 | 0.1 – 4.5 | 0.6 | 0.1 - 5.0 |
| | DTaP-IPV/Hib (4) | 0.9 | 0.2 – 4.1 | 1.1 | 0.2 - 5.4 |
| | MV2 | 0.9 | 0.2 – 4.1 | 1.1 | 0.2 - 5.3 |
| | | | | | |

**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema*

**Appendix 8: Table: Association between immunisation status and self-reported eczema, asthma and hay fever for urban participants**

| Outcome | Vaccine | OR | 95% CI | *adjusted OR | 95%CI |
|---|---|---|---|---|---|
| **Self-reported** | BCG | 1 | | | |
| **asthma** | OPV(0) | 0.6 | 0.1 - 5.5 | 0.5 | 0.1 - 4.6 |
| | OPV(1) | 0.4 | 0.04 - 3.7 | 0.3 | 0.03 - 3.1 |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 0.4 | 0.04 - 3.7 | 0.3 | 0.03 - 2.5 |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 1 | | | |
| | DTaP-IPV/Hib (4) | 0.8 | 0.4 – 1.9 | 0.7 | 0.3 - 1.7 |
| | MV2 | 0.7 | 0.3 – 1.5 | 0.6 | 0.2 - 1.4 |
| **Self-reported** | BCG | 1 | | | |
| **Hay fever** | OPV(0) | 0.7 | 0.1 - 4 | 0.7 | 0.1 - 3.7 |
| | OPV(1) | 0.4 | 0.1 – 2.6 | 0.3 | 0.1 - 2.4 |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1 | | | |
| | DTaP-IPV/Hib (3) | 1.09 | 0.1 - 10.5 | 0.9 | 0.1 – 8.8 |
| | HBV(3) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 0.96 | 0.3 - 3.7 | 1.0 | 0.3 – 4.0 |
| | PCV(3) | 0.99 | 0.3 - 3.2 | 1.0 | 0.3 - 3.3 |
| | DTaP-IPV/Hib (4) | 0.6 | 0.3 – 1.1 | 0.6 | 0.3 - 1.1 |
| | MV2 | 0.7 | 0.4 – 1.3 | 0.7 | 0.4 - 1.3 |
| **Self-reported** | BCG | 1 | | | |
| **eczema** | OPV(0) | 0.7 | 0.1 - 3.6 | 0.6 | 0.1 - 3.4 |
| | OPV(1) | 0.97 | 0.1 – 9.4 | 1.0 | 0.1 - 10 |
| | RV (1) | 1 | | | |
| | DTaP-IPV/Hib (1) | 1 | | | |
| | HBV(1) | 1 | | | |

| | | | | | |
|---|---|---|---|---|---|
| | PCV(1) | 1 | | | |
| | DTaP-IPV/Hib (2) | 1 | | | |
| | HBV(2) | 1 | | | |
| | RV (2) | 1.3 | 0.1 – 11.7 | 1.4 | 0.2 - 13 |
| | DTaP-IPV/Hib (3) | 0.97 | 0.1 - 9.4 | 1.0 | 0.1 - 10 |
| | HBV(3) | 0.7 | 0.06 - 7.2 | 0.7 | 0.1 - 7.7 |
| | PCV(2) | 1 | | | |
| | MV(1) | 0.9 | 0.2 - 3.3 | 0.8 | 0.2 - 3.1 |
| | PCV(3) | 0.9 | 0.3 - 2.8 | 0.9 | 0.3 - 2.8 |
| | DTaP-IPV/Hib (4) | 1.4 | 0.7 – 2.9 | 1.2 | 0.6 - 2.6 |
| | MV2 | 1.6 | 0.8 – 3.5 | 1.4 | 0.7 - 3.1 |
| | | | | | |

**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema. Self-reported eczema odds ratio adjusted for age at enrolment, gender, breast fed and number of older children in the house.*

## Appendix 9: Table: Associations between immunisation status and SPT≥ 1mm, ≥ 3mm and ≥ 7mm in rural participants

| Outcome | Vaccine | OR | 95% CI | *adjusted OR | 95%CI |
|---|---|---|---|---|---|
| **SPT ≥ 1** | OPV(1) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 0.7 | 0.1 - 5.8 | 0.6 | 0.1 - 5.4 |
| | DTaP-IPV/Hib (4) | 1.4 | 0.2 - 11.1 | 1.4 | 0.2 - 12.0 |
| | MV2 | 1.4 | 0.2 - 11.1 | 1.4 | 0.2 - 11.7 |
| **SPT ≥ 3** | OPV(1) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 1 | | | |
| | DTaP-IPV/Hib (4) | 1 | | | |
| | MV2 | 1 | | | |
| **SPT ≥ 7** | OPV(1) | 1 | | | |
| | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 1 | | | |
| | DTaP-IPV/Hib (4) | 1 | | | |
| | MV2 | 1 | | | |
| | | | | | |

**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema*

**Appendix 10: Table: Association between immunisation status and self-reported eczema, asthma and hay fever for rural participants**

| Outcome | Vaccine | OR | 95% CI | *adjusted OR | 95%CI |
|---|---|---|---|---|---|
| **Self-reported** | OPV(1) | 1 | | | |
| **asthma** | PCV(2) | 1 | | | |
| | MV(1) | 1 | | | |
| | PCV(3) | 1 | | | |
| | DTaP-IPV/Hib (4) | 1 | | | |
| | MV2 | 1 | | | |
| **Self-reported** | OPV(1) | 1 | | | |
| **Hay fever** | PCV(2) | 1 | | | |
| | MV(1) | 0.3 | 0.04 - 2.7 | 0.4 | 0.1 - 3.5 |
| | PCV(3) | 0.5 | 0.1 - 3.7 | 0.5 | 0.1 - 4.0 |
| | DTaP-IPV/Hib (4) | 0.9 | 0.1 - 7.3 | 0.9 | 0.1 - 8.1 |
| | MV2 | 0.9 | 0.1 - 7.3 | 0.8 | 0.1 - 7.7 |
| **Self-reported** | OPV(1) | 1 | | | |
| **eczema** | PCV(2) | 1 | | | |
| | MV(1) | 0.2 | 0.02 - 1.6 | 0.2 | 0.03 - 2.4 |
| | PCV(3) | 0.3 | 0.03 - 2.3 | 0.3 | 0.03 - 2.9 |
| | DTaP-IPV/Hib (4) | 0.4 | 0.04 - 3.6 | 0.2 | 0.02 - 2.4 |
| | MV2 | 0.4 | 0.04 - 3.6 | 0.3 | 0.02 - 2.8 |

**odds ratios adjusted for age at enrolment, gender, breast fed, number of older children in the house and clinically diagnosed eczema. Self-reported eczema odds ratio adjusted for age at enrolment, gender, breast fed and number of older children in the house.*